

ABORTIVE T5 BACTERIOPHAGE INFECTIONS
OF Escherichia coli CONTAINING
THE COLICINOGENIC FACTOR, Col1b

By

JERRY GLENN

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1979

To Steven E. Ross and the late James W. Dunlop -
Their accomplishments have given me inspiration, and
their advice and encouragement have given me direction.

ACKNOWLEDGEMENTS

I acknowledge the help and support of my parents and my wife. Without them, this dissertation would not be.

Also, I want to express my gratitude to the Department of Surgery of the University of Florida, College of Medicine. They have given me the freedom to pursue an extremely unconventional surgical training program.

Finally, I thank the people who have guided me in learning to answer questions in a scientific manner. My advisory committee -- Drs. Donna Duckworth, Kenneth Berns, George Gifford, and Carl Feldherr -- was quite helpful. In particular, I thank Donna Duckworth for giving me an idea whose time had come and for guiding me in the right direction. Also, discussions with Kenneth Berns during Dr. Duckworth's sabbatical year were sustaining. Drs. William Holloman, Nicholas Muzyczka, and Thomas Pinkerton contributed numerous suggestions. Glenda Dunn gave me invaluable technical instruction and assistance.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABBREVIATIONS USED	ix
ABSTRACT	x
INTRODUCTION	1
Abortive Infections Mediated by the	
ColIb Factor	2
MATERIALS AND METHOOS.	9
Organisms	9
Media and Growth of Bacteria	9
Colicin Production	11
Phage Growth	11
Phage Infections	12
Macromolecular Synthesis	12
Oye-Buoyant Density Equilibrium	
Centrifugation of Bacterial ONA.	13
Host-Cell DNA Breakdown	15
Proline and Glutamine Uptake	15
α -Methylglucoside (α MG) Uptake	16
Fluorescence Experiments	16
Potassium Efflux	17
Attempts to Prevent Abortive of T5	
Infection in ColIb ⁺ Hosts.	18
Gel Analysis	18
Statistical Methods.	19
Materials	20
RESULTS	21
Macromolecular Synthesis in T5 and T5h12 ⁻	
Infections of RM 42, RM 43, and RM 39.	21
Confirmation that RM 39 Contains	
Plasmid DNA.	28
Confirmation that T5h12 ⁻ Infections	
Begin Promptly	31
Amino Acid Accumulation in T5 and T5h12 ⁻	
Infections of RM 42, RM 43, and RM 39.	31
Confirmation that Inhibited Proline and	
Glutamine Accumulation is Not Due Solely	
to Inhibited Protein Synthesis	42
α -Methylglucoside Accumulation in T5 and	
T5h12 ⁻ Infection of RM 42, RM 43, and	
RM 39.	42
Glucose Incorporation into Macromolecules	
in T5 and T5h12 ⁻ Infection of RM 42,	
RM 43, and RM 39	44

Fluorescence Intensity of NPN During T5 and T5 Δ 12 ⁻ Infection of RM 42, RM 43, and RM 39	50
Potassium Efflux During T5 Infection of RM 42, RM 43, and RM 39	53
Attempts to Prevent Abortion of T5 Infection of Col1b-Containing Cells.	53
Macromolecular Synthesis in T5 Wild-Type and T5 Δ 1 ⁻ Infection of RM 42, RM 43, and RM 39.	57
Proline, Glutamine, and MG Accumulation in T5 Wild-Type and T5 Δ 16d-Infected RM 42, RM 43, and RM 39.	59
Fluorescence of Membrane-Bound NPN During T5 Δ 1 ⁻ Infections.	71
Potassium Efflux During T5 Δ 16d Infections of RM 43	71
Absence of Host DNA Breakdown During T5 Δ 16d or T5 Δ 127 Infection	75
Gel Analysis of Phage Proteins	75
DISCUSSION	81
BIBLIOGRAPHY	93
BIOGRAPHICAL SKETCH.	101

LIST OF TABLES

1. Characterization of Bacteria and Phage	10
2. Proline Accumulation (in 60 seconds) by Infected RM 42, RM 43, and RM 39.	37
3. Glutamine Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39.	41
4. Proline and Glutamine Accumulation in Uninfected and Infected RM 43 After 1 Minute or 15 Minutes of Incubation with Chloramphenicol	43
5. α -Methylglucoside Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39.	47
6. Potassium Efflux from T5-Infected RM 42, RM 43, and RM 39.	56
7. Attempts to Prevent Abortion of T5 Infection of ColIb ⁺ Hosts.	58
8. Proline Accumulation (in 60 seconds) by Infected RM 42, RM 43, and RM 39.	66
9. Glutamine Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39.	68
10. α -Methylglucoside Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39.	70
11. Potassium Efflux from T5A1 ⁻ -Infected RM 42 and RM 43.	73

LIST OF FIGURES

1. Uridine Incorporation into Acid-insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39	22
2. Tyrosine Incorporation into Acid-insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39	24
3. Proline Incorporation into Acid-insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39	27
4. Ethidium Bromide-Cesium Chloride Gradients of DNA Extracted from RM 42, RM 43, and RM 39	30
5. Host DNA Breakdown After T5h12 ⁻ -Infection of RM 42, RM 43, and RM 39.	32
6. Proline Accumulation in 60 Seconds by Infected RM 42, RM 43, and RM 39.	36
7. Glutamine Accumulation in 90 Seconds by Infected RM 42, RM 43, and RM 39	40
8. α -Methylglucoside Accumulation in 90 Seconds by Infected RM 42, RM 43, and RM 39	46
9. Glucose Incorporation into Acid-insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39	48
10. Fluorescence Intensity of N-phenyl-1- naphthylamine During T5 and T5h12 ⁻ - Infections of RM 42, RM 43, and RM 39	52
11. Potassium Efflux from T5-Infected RM 42, RM 43, and RM 39.	55
12. Uridine Incorporation into Acid-insoluble Macromolecules in Uninfected and T5A1 ⁻ - Infected RM 42, RM 43, and RM 39.	60
13. Tyrosine Incorporation into Acid-insoluble Macromolecules in Uninfected and T5A1 ⁻ - Infected RM 42, RM 43, and RM 39.	62

14.	Proline Accumulation in 60 Seconds by T5A1 ⁻ -Infected RM 42, RM 43, and RM 39.	65
15.	Glutamine Accumulation in 90 Seconds by T5A1 ⁻ -Infected RM 42, RM 43, and RM 39.	67
16.	α -Methylglucoside Accumulation in 90 Seconds by T5A1 ⁻ -Infected RM 42, RM 43, and RM 39.	69
17.	Fluorescence Intensity of N-phenyl-1- naphthylamine During T5A1 ⁻ -Infections of RM 42, RM 43, and RM 39	72
18.	Potassium Efflux from T5A1 ⁻ -Infected RM 42 and RM 43.	74
19.	Absence of Host DNA Breakdown During T5A1 ⁻ -Infections of RM 42, RM 43, and RM 39.	76
20.	Gel Analysis of Phage-Induced Proteins During T5 and T5A1 ⁻ -Infections of RM 42 and RM 43.	80

ABBREVIATIONS USED

α MG	-- α -methylglucoside (α -methyl-D-glucopyranoside)
DCCD	-- N,N'-dicyclohexylcarbodiimide
EDTA	-- ethylenediamine tetraacetate
NPN	-- N-phenyl-1-naphthylamine
ONPG	-- <u>o</u> -nitrophenylgalactoside
SDS	-- sodium dodecyl sulfate
SV40	-- simian virus 40
TCA	-- trichloroacetic acid
TEMED	-- N,N,N',N'-tetra-methyl-ethylenediamine
TES buffer	-- Tris-EDTA-saline buffer
TMG	-- thio- β -methyl-D-galactoside

Abstract of Dissertation Presented to the Graduate Council
of the University of Florida in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy

ABORTIVE T5 BACTERIOPHAGE INFECTIONS
OF *Escherichia coli* CONTAINING
THE COLICINOGENTIC FACTOR, ColIb

By

Jerry Glenn

June 1979

Chairperson: Donna H. Duckworth

Major Department: Immunology and Medical Microbiology

When bacteriophage T5 infect *Escherichia coli* containing the colicinogenic factor, ColIb, the infections are abortive. RNA and protein syntheses stop abruptly and simultaneously at about 12 minutes after adsorption, and no progeny phage are produced. In the current work, I have shown that numerous changes in membrane function appear at nearly the same time as cessation of macromolecular syntheses. These alterations include inhibition of glutamine and proline uptake, stimulation of α -methylglucoside uptake, increased fluorescence intensity emitted by a membrane-binding probe, and efflux of preloaded potassium ions. The combination of results suggests that the infectious process is halted because of membrane depolarization. The same pattern of pathophysiological changes occurs during infections of ColIb⁺ hosts by T5 mutants deficient in second-step transfer of DNA, indicating that no early or late phage gene expression is necessary to elicit the abortive response.

INTRODUCTION

Extrachromosomal elements of bacteria carry from cell to cell a tremendous variety of genetic determinants. At least 30 types of lysogenized phage are found among coliforms (26), and over 250 kinds of plasmids are established in E. coli alone (83). Actually, such figures might grossly underestimate the true numbers, since modifications and rearrangements of nucleotide sequences within any single cell probably continuously yield new genetic combinations (22,89). These novel structures efficiently pass to other bacteria -- even to unrelated bacteria. Then, the process is repeated, implementing rapid change in the genetic complement of bacterial populations.

Extrachromosomal elements are eminently suited, therefore, to serve as instruments for bacterial adaptation, and they do in fact often provide strong survival advantage to the cells in which they reside (89). In certain Enterobacterial strains, for example, some types of extrachromosomal elements enable their hosts to prevent replication of particular virulent bacteriophages. This can be accomplished in several ways. Some elements carry genetic determinants that alter specific viral receptors, making the cell resistant to the phage which normally adsorb to that site (1). Another mechanism is superinfection exclusion (2,3,31). Here, adsorption is normal. This term describes the ability of one phage -- the extrachromosomal element in this case -- to prevent entry of DNA from a second phage that is subsequently adsorbed. The genome of the

superinfecting virus is trapped within the cell envelope, preventing any phage gene expression. Yet another way that some genetic elements circumvent viral replication is by directing the synthesis of restriction endonucleases (72). These enzymes break down incoming DNA that is unmodified at specific restriction sites. Finally, there is abortive infection, during which adsorption and DNA entry are normal, and the viral genome remains intact. The initial stages of viral gene expression occur as usual, but the presence of a lysogenized phage or a plasmid in some way prevents the infecting virus from producing progeny.

Of these four mechanisms -- resistance, superinfection exclusion, restriction, and abortive infection -- abortive infection remains the least understood. This is the case despite extensive study of several abortive systems, among which are T7 infections of F (fertility factor)-containing *E. coli* (11,12,13,23,24,68,76), T-even rII mutant infections of λ lysogens (14,34,39,92), and T-even or T5 infections of P2 lysogens (8,9,10,35).

The long-term objective of my research is to elucidate the mechanism underlying the abortive infection which occurs after T5, or its close relative BF23, infects *E. coli* containing the colicinogenic factor, ColIb. Studies described herein answer several questions that are important steps toward realizing this goal.

Abortive Infections Mediated by the ColIb Factor

Strains of *E. coli* carrying the colicinogenic factor, ColIb, are nonpermissive hosts for bacteriophage BF23 (80,97). However, bacteria containing the F-factor, the λ prophage, or the P1 prophage are able

to support replication of this phage. Likewise, BF23 can replicate in the presence of ColB, ColV, ColE1-30, ColE2-P9, and ColIa. Thus, the viral infection is not aborted because of the presence of extra-chromosomal DNA in general; rather, specific genetic determinants on the plasmid are involved.

It is not surprising that T5 shares BF23's inability to grow in ColIb-containing cells (80). This characteristic is just one of many the two phages have in common (reviewed in 87). They also have colinear maps and undergo phenotypic mixing, gene product substitution, and genetic recombination.

Productive BF23 and T5 infections are very similar as well. Both viruses are unusual in that they inject their genomes in two steps (59,60,61,62). The first-step-transfer DNA, constituting only 8% of the whole, codes exclusively for class I (pre-early) proteins (70). These proteins are synthesized from about 1 minute until about 10 minutes after infection. One of them, the A1 gene product, is required for host DNA degradation (62,71). The A1 polypeptide and also the A2 gene product are required for entry of the remaining second-step-transfer DNA. Carrying all class II (early) and class III (late) genes, the other 92% of the genome enters the host at about 4 minutes after infection, several minutes before early protein synthesis begins (70). The early proteins are necessary for phage DNA replication, which begins about 9 minutes into the infectious process (25). Late proteins, the structural proteins included, first appear at about 13 minutes and are synthesized until lysis, approximately 1 hour after infection (70).

Abortive infections begin in the same way, but become manifestly abnormal after a short time (79). Phage DNA entry occurs normally, and the infecting genome remains intact (79,97). Pre-early gene expression proceeds as usual, resulting in degradation of the bacterial genome and death of the host cell. Then, at about 8 to 12 minutes after adsorption, incorporation of RNA and protein precursors into macromolecules stops. Little early protein synthesis occurs, and no late proteins appear. Since early proteins are required for phage DNA synthesis (69), it is not surprising that phage DNA replication is prevented. Finally, some laboratories observe rapid cell lysis of infected nonpermissive cells 15 to 30 minutes after phage protein synthesis begins (80).

A few phage circumvent the abortive process, producing plaques at an efficiency of plating about 10^{-6} relative to that on noncolicinogenic hosts (74,80). These host-range (h^{-}) mutants, which are recessive to wild-type in mixed infections, have a mutation in a pre-early gene (5,74). In T5, the mutation lies in gene A3, and the mutants fail to produce a small protein with a molecular weight of 12,000 (98). The T5 A3 gene product is thought to be identical to PE5 seen on polyacrylamide gels after electrophoresis of infected-cell extracts (D. J. McCorquodale, personal communication). Similarly, BF23 host-range mutants have a mutation in gene P3 (corresponding to A3 of T5 on the colinear maps). Furthermore, the electrophoretic mobility of PE5 derived from cells infected with BF23 h^{-} mutants is altered, consistent with the suggestion that PE5 is the P3 gene product. Hence, it appears that the A3 or P3 gene product must be present to elicit the abortive response.

It has been proposed that early protein synthesis is required in abortive T5 infections (45). It had been shown that if T5 infects cells in the presence of very low calcium concentrations, only pre-early proteins are expressed (77,78). When additional calcium is added later, however, early RNA and proteins quickly appear. If Colib⁺ hosts are infected with T5 in the presence of little calcium, allowed to synthesize only pre-early proteins for 12 minutes, then supplemented with additional calcium, RNA synthesis proceeds for only a short time thereafter; i.e., the infection aborts soon after calcium supplementation (45). If the same experiment is performed, except that chloramphenicol is added just prior to calcium addition, RNA synthesis continues much longer than when chloramphenicol is not added. The implication is that chloramphenicol, in the second case, prevents synthesis of an early protein(s) necessary for activating the abortive mechanism. At least one alternative explanation, however, is that a low calcium concentration present at the time of infection might also slow production of pre-early proteins (69). This could be expected to inhibit production of the A3 gene product and might delay cessation of macromolecular synthesis.

To determine if any early protein synthesis is indeed necessary to halt the infectious process, I have taken another approach using T5A1⁻ mutants. Because a functional A1 protein is lacking, these phage can synthesize only pre-early proteins; second-step DNA transfer does not occur (62,71). I will show here that the abortive response appears despite the absence of early proteins, suggesting that the only viral protein required in T5 abortive infection is the A3 gene product.

In addition to the phage's own contribution, host-determined factors are involved in abortive infection. One-step growth curves indicate that roughly 5% of the cells in a ColIb⁺ population will support phage replication, though the phage yield per infective center is decreased by 70-85% (75,80). Since the phage that do manage to grow do not grow well upon reinfection of ColIb-containing cells, these phages are not host-range mutants; rather, their growth depends on a low level of permissiveness in a few cells among the host population. The degree of T5 inhibition is also dependent to some extent upon the particular host strain carrying the plasmid (79). For example, the yield of T5 infectious centers on E. coli W3110 (ColIb) is similar to that if T5 is grown on W3110 polA1 (ColIb). The average phage yield per infective center, however, is 5-fold greater on the strain with normal ability to produce host DNA polymerase I.

Furthermore, some ColIb-containing strains are permissive because of mutations on chromosomal or plasmid DNA (50,69, Richard Moyer, personal communication). Genetic analysis of these strains reveals that mutations at two chromosomal loci are necessary to express a fully permissive phenotype. The first locus, designated cmrA (ColIb-mediated resistance) on the E. coli map, is 91% cotransducible with rspE and is proximal to aroE. The second, cmrB, is 75% cotransducible with rspL and is distal to aroE. Other strains are permissive because of plasmid-borne mutations, but these have not been mapped. Thus, taken together, the studies indicate that contributions by plasmid, host, and phage are all necessary to produce an abortive infection.

To determine how the various factors interact to arrest the infection is a complex problem. It had been hypothesized that the primary defect is in transcription (79). Fully active RNA polymerase is recoverable from infected ColIb-containing cells, however, indicating that this enzyme is not the target (99). Alterations of the DNA template have also been studied to see if there are any which could explain cessation of gene expression. Packaged DNA is nicked, but the genome is ligated soon after infection (45). Only in productive infections, however, is the DNA subsequently renicked, suggesting that the transcriptional program during abortive infection could be altered as a result. Since ligated DNA is transcribed normally in an in vitro system (54), though, the relevance of the finding is questionable. A direct approach would be to determine if DNA extracted from abortively infected cells can direct in vitro transcription, but this has not been done.

It has also been proposed that the primary defect is at the translational level (50). The cmrA locus maps near the gene coding for a ribosomal protein. This suggests that the ribosomal apparatus might be implicated, but no study has determined if extracts from abortively infected cells can support in vitro translation. Thus, while no sound basis for a primary transcriptional or translational defect has been found, the studies looking for such abnormalities have not been exhaustive and cannot, therefore, rule out these possibilities.

Since it has previously been hypothesized that membrane dysfunction causes the abortion of T7 infections of male E. coli (12,23)

and of T-even rII mutant infections of λ lysogens (34,39), another line of investigation has been to examine changes in the cell envelope during abortive T5 infection. Infected nonpermissive cells become sensitive to sodium dodecyl sulfate (SDS)-induced lysis, unlike their infected ColIb⁻ counterparts (19). Although indicative of structural alterations in the host's outer membrane which allows the detergent to reach the inner membrane, this change occurs later than the cessation of macromolecular synthesis. As mentioned previously, some laboratories also note early abortive lysis (80), but the meaning of this event is not clear either. No progeny phage are produced even when premature lysis is prevented by stabilizing the host cells in medium of high osmolarity. Furthermore, our laboratory does not observe the spontaneous lysis (19).

I have looked more closely at membrane function during T5 infections of ColIb⁺ hosts to determine if physiological alterations of the membrane could possibly account for the abortive response. The results suggest that host membrane depolarization is the primary event leading to cessation of the T5 infectious process.

MATERIALS AND METHODS

Organisms

The bacterial strains used for most experiments are characterized in Table 1. Richard Moyer supplied our laboratory with three strains which are isogenic. RM 42 contains no plasmid; RM 43 bears wild-type ColIb-P9; RM 39 has a mutant ColIb plasmid which renders the host permissive for T5 (Richard Moyer, personal communication). Other strains were occasionally used, as indicated. E. coli was originally obtained from M. J. Bessman and maintained for many years in Donna Duckworth's laboratory, whereas E. coli C(HF4704) was taken from a stock maintained by William Holloman. E. coli CR63 was the gift of M. L. Dirksen.

Rolf Benzinger provided wild-type bacteriophage T5; T5h12⁻, T5am16d, and T5amH27 were obtained from D. J. McCorquodale. Relative plating efficiencies of the phage stocks on the various bacterial strains are also shown in Table 1. T5h12⁻ has a mutation which allows it to replicate in ColIb⁺ cells (74), whereas T5am16d and T5amH27 each have an amber mutation in the A1 gene (5).

Media and Growth of Bacteria

The growth medium used for most experiments was M9 phosphate-buffered, balanced salt solution (46), supplemented with glucose (0.5%), yeast extract (0.05%), CaCl₂ (5×10^{-4} M), and thymine (50 µg per ml). Other additives were present as indicated in the description of each experiment. Unless otherwise indicated, bacteria were grown from a

TABLE 1. Characterization of Bacteria and Phage

E. coli Strain	Genotype	Colicin Production	Plating Efficiency Relative to CR63(su ⁺)			
			T5 Wild-type(T5wt)	T5h12 ⁻	T5am16d	T5amh127
RM 42	W3110 (thy ⁻ , ColI ^{R1})	-	1	1	10 ⁻⁵	10 ⁻⁵
RM 43	W3110 (tny ⁻ , ColI ^R , ColIb-p9)	+	10 ⁻⁷	0.5	<10 ⁻⁹	<10 ⁻⁹
RM 39	W3110 (thy ⁻ , ColI ^R , ColIb-p9h ⁻²)	+	1	3	10 ⁻⁵	10 ⁻⁵

¹ColI^R indicated resistance to the external action of Colicin Ib.

²ColIb-p9h⁻ indicates a mutant ColIb factor which allows T5 replication.

5% inoculum of an overnight culture. Growth was followed by monitoring turbidity on a Klett-Summerson colorimeter (660 nm filter), which had been previously calibrated to numbers of bacterial colony formers.

Potassium efflux experiments were performed using dilute tryptone broth (4 g tryptone and 2.5 g NaCl per liter of medium) as growth medium. Hershey broth (8 g of nutrient broth, 5 g of peptone, 5 g of NaCl per liter) was employed for some purposes, where indicated. Growth in these media was followed in the manner outlined above.

Finally, Tris-buffered medium was used. Per liter, this medium contains 2.0 g of NH_4Cl , 5.0 g of NaCl, 0.4 g of KCl, 0.01 g of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.02 g of Na_2SO_4 , 5.0 g of Casamino acids, 2.5 ml of 80% glycerol, 250 mg of thiamine, and 100 ml of Tris (1 M, pH 7.3).

Colicin Production

To determine if a particular bacterial strain produced a colicin, I used the method of Fredericq (38). The cells were grown in Hershey broth for 48 hours. Then, chloroform (1%) was added, and the suspension was agitated for 10 seconds. Thereafter, the suspension was centrifuged at 10,000 rpm for 10 minutes on an SS-34 rotor in a Sorvall (RC-5). The supernatant (0.005 ml) was spotted on sensitive indicator lawns of *E. coli* B and *E. coli* C. If a zone of clearing appeared after overnight incubation of the plates, the strain was called a colicin-producer. Results are indicated in Table 1.

Phage Growth

Phage stocks were prepared in one of two ways: by the confluent lysis technique of Adams (1), or by recovering phage from Hershey broth by polyethylene glycol precipitation (105). No differences in results were obtained using the different types of phage preparation.

Phage Infections

Bacterial hosts were grown, as outlined above, to a concentration of approximately 6×10^8 cells per ml. The bacteria were then centrifuged at 4°C for 10 minutes at 10,000 rpm in an SS-34 rotor on Sorvall (RC-5), and resuspended in 1/10th volume of fresh growth medium. Phage were added at a multiplicity of infection of 10 (except where indicated otherwise), and after 1 minute, the bacteria-virus mixture was rediluted in prewarmed growth medium to 6×10^8 cells per ml. The time of redilution was called time zero.

Macromolecular Synthesis

RNA synthesis was monitored by measuring incorporation of [^3H]-uridine into acid-insoluble material at different times after infection. Bacteria were grown in basic growth medium, with 50 μg of uridine added per ml of medium. Bacteria were centrifuged, diluted, infected, and rediluted as outlined above. At time zero, [^3H] uridine (1 μCi per ml, 5 μCi per μmole) was added and, at indicated intervals thereafter, 0.9 ml aliquots were removed and added to 0.1 ml of cold 50% trichloroacetic acid (TCA). Of this mixture, 0.4 ml was filtered through Whatman glass fiber filters (GF/F) and washed with 15 ml of cold 5% TCA. The filters were dried in an oven at 60°C for about 1 hour, and counted in a toluene-based liquid scintillation fluid using a Beckman LS200 counter. The results represent total (cumulative) counts per minute incorporated into samples of approximately 2.2×10^8 bacteria.

Protein synthesis was measured in the same way, except that amino acids were used. When tyrosine was employed, cold tyrosine (25 μg per ml) was added to basic growth medium, and [^3H] tyrosine

(1 μCi per ml, 7.2 μCi per μmole) was added at time zero. When proline incorporation was measured, cold proline (25 μg per ml) was added to basic growth medium, and [^3H] proline (1 μCi per ml, 4.8 μCi per μmole) was added at time zero. Experiments to measure incorporation of glucose were also performed as above, adding [^{14}C] glucose (0.5 μCi per ml, 0.1 μCi per μmole) at time zero.

Scintillation fluid consisted to toluene (1 gallon), 19 g of PPO (2,5-diphenyloxazole), and 1.14 g of POPOP [1,4-bis-(5-phenyloxazsyl) benzene]. A magnetic stirring bar agitated a newly made mixture overnight.

Dye-Buoyant Density Equilibrium Centrifugation of Bacterial DNA

Bacterial DNA was labeled, extracted, and banded on ethidium bromide-caesium chloride gradients according to a modification of the method of Clewell and Helinski (20,21).

Tris-buffered growth medium was used to grow bacteria from a 1:100 dilution of an overnight culture until the turbidity reached 115 Klett units (660 nm filter). RM 42 and RM 39 were grown in 15 ml of medium, whereas 30 ml of an RM 43 culture was grown. RM 42 and RM 39 were incubated in the presence of [^{14}C] thymidine (50 μCi per ml, 1 μCi per 4 μg of thymidine); [^3H] thymidine (50 μCi per ml, 1 μCi per 4 μg of thymidine) was used to label RM 43 DNA. After cells had reached the desired concentration, 15 ml of the RM 42 culture was mixed with 15 ml of the RM 43 culture, and 15 ml of the RM 34 culture was mixed with 15 ml of the RM 43 culture.

Each tube, containing a total of 30 ml of bacterial suspension, was spun at 10,000 rpm in an SS-34 rotor in a Sorvall (RC-5). Each

pellet was resuspended in 1 ml of cold 25% sucrose in Tris (0.05 M, pH 8). Lysozyme (0.2 ml of a solution, 5 mg per ml in Tris [0.25 M, pH 8]) was added, and the suspension was maintained at 0°C for 5 minutes. Thereafter, ethylenediamine tetraacetate (EDTA) (0.4 ml of an aqueous solution, 0.25 M adjusted to pH 8) was added subsequently and, with occasional swirling, maintained for another 5 minutes at 0°C. Then, 1.6 ml of a detergent solution (1% Brij 58, 0.4% sodium deoxycholate, 0.0625 M EDTA, 0.05 M Tris, pH 8) was added to the suspension, and the mixture was maintained at 0°C until cell lysis, 3 to 5 minutes later. The lysate was spun at 2°C for 25 minutes at 20,000 rpm in an SS-34 rotor on a Sorvall (RC-5). The supernatant should only contain about 5% of the original quantity of chromosomal DNA and is called the cleared lysate.

Each of the cleared lysates was diluted to 12 ml in TES buffer (0.05 M NaCl, 0.005 M EDTA, 0.03 M Tris, pH 8), and 3 ml of an ethidium bromide solution (1 mg of ethidium bromide in 1 ml of TES buffer) was added. Then, CsCl was added in a quantity sufficient to bring the refractive index to 1.3886. Each mixture was spun in a Ti-60 fixed angle rotor at 32,000 rpm at 4°C for 60 hours.

An 18 gauge needle was inserted into the bottom of each centrifuge tube, and 0.25 ml samples were recovered. The refractive index of representative samples was determined. Each sample was then mixed with 0.05 ml of 50% trichloroacetic acid, and 0.15 ml of this mixture was filtered over Whatman glass fiber filters (GF/F). Each filter was washed with 15 ml of 5% TCA, placed in a glass vial, and dried in an oven at 60°C for 1 hour. A toluene-based scintillation fluor was added (5 ml in each vial), and the scintillations per minute were

determined on a Beckman LS200 counter using the narrow [^3H]-window and the [^{14}C]-window purchased from Beckman.

Host-Cell DNA Breakdown

Bacteria were grown as previously described in basic growth medium (defined above) modified by having thymidine (25 μg per ml) in place of thymine. The medium also contained [^3H] thymidine (0.4 μCi per ml; 1 μCi per 50 μg of thymidine). When the cells reached the appropriate concentration, they were spun, washed twice in medium without labeled thymidine, concentrated, infected, and rediluted as described previously. Thereafter, 0.9 ml samples were removed at the indicated intervals and were added to 0.1 ml of 50% cold TCA. Of this, 0.4 ml samples were filtered over Whatman glass fiber filters (GF/C) and washed with 15 ml of 5% TCA. The filters were placed in glass vials and dried for 1 hour in an oven at 60°C. Scintillations per minute in a toluene-based liquid scintillation fluid were counted on a Beckman LS200 counter.

Proline and Glutamine Uptake

In these experiments, the amount of amino acid taken up by the cell in a 30-, 60-, or 90-second pulse is measured at various times after infection. Bacteria were grown in basic growth medium, spun, concentrated, infected, and rediluted as above. At the indicated times, 2 ml samples were removed, and chloramphenicol was added, yielding a final concentration of 100 μg per ml. One minute later, 0.9 ml of this mixture was added to 0.1 ml of the labeled amino acid solution. In the proline assay, 1 μCi [^3H] proline (25 μCi per μmole) was present, while in the glutamine experiments, 0.5 μCi of

[^{14}C] glutamine (5 μCi per μmole) was present. At indicated intervals after infected bacteria were added to the labeled amino acid, 0.4 ml samples were removed, filtered on Whatman glass fiber filters (GF/F), and washed with 8 ml of M9. A positive control was done using uninfected cells; a negative control was done using cells treated for 15 minutes with NaN_3 (1%) prior to addition of chloramphenicol. The results are presented here as percentage of uptake in infected cells, relative to that of uninfected controls.

α -Methylglucoside (αMG) Uptake

α -Methylglucoside uptake experiments utilized bacteria grown and infected in basic growth medium, as outlined above. At times indicated, 1 ml of the sample was removed and spun in an Eppendorf 3200 Centrifuge for approximately 30 seconds, resuspended in an equal volume of M9, and spun again. After the second spin, the bacteria were resuspended in basic growth medium, modified by having only 18 μg glucose per ml of medium. Of this suspension, 0.9 ml was added to 0.1 ml of [^{14}C] MG solution (1 μCi per ml, 184 μCi per mole). At indicated intervals, 0.4 ml aliquots were removed and filtered over Whatman glass fiber filters (GF/F) and washed with 8 ml of M9. The filters were dried and counted. A positive uninfected control was done; a negative control was done using cells treated for 15 minutes prior to the assay with NaF (0.07 M) and NaN_3 (1%). The results are presented as percentage of uptake, relative to uninfected controls.

Fluorescence Experiments

Fluorescence intensity was measured using a Perkin-Elmer MPF-2A scanning fluorimeter equipped with a temperature-controlled chamber.

The instrument's output was corrected for wavelength variable response by means of a rhodamine B quantum counter, and its monochromators were calibrated against the xenon line emission spectrum.

From recrystallized N-phenyl-1-naphthylamine (NPN) a working stock (20 mM in methanol) was prepared. Cells were grown, concentrated, and infected as described previously, except that the multiplicity of infection was 5. At time zero, the infected cells were resuspended in prewarmed (37°C) growth medium containing 10 μ M NPN. Three milliliter of the infected-cell samples was placed in quartz cuvettes and inserted into the 37°C chamber.

Fluorescence intensity was measured in arbitrary units and recorded over time after infection. Excitation wavelength was 352nm with a bandwidth of 5nm. The emission wavelength was 410nm with a bandwidth of 10nm.

Potassium Efflux

Dilute tryptone broth was used as growth medium. This medium contains a low concentration of potassium, making it easier to load cells with radiolabeled potassium (94,101).

Bacteria were grown for several generations in the presence of 0.1-0.2 mCi of ^{42}K per ml (^{42}K in the form of KCl in aqueous solution, 0.18 mCi per mg K). When the cells reached a concentration of 6×10^8 bacteria per ml, they were spun, concentrated, and infected as usual (a portion of cells was not infected). At intervals, aliquots of uninfected and infected cells were removed, filtered over glass fiber filters (GF/F), and washed with 8 ml of dilute tryptone broth. Cerenkov emissions were counted using the [^3H] channel on the Beckman

LS200 counter. The results represent the amount of residual ^{42}K remaining inside infected cells, relative to the amount remaining inside uninfected cells taken at the same time point (expressed as a percentage).

Attempts to Prevent Abortion of T5 Infection in ColIb⁺ Hosts

Cells were grown and infected, in 1% tryptone broth with Tris (5 mM, pH 7.2), as outlined above. Various additives were present as indicated in Table 7. The final concentrations of the additives were as follows: (a) potassium, 100 mM (b) magnesium, 80 mM (c) sodium, 80 mM (d) sucrose, 300 mM (e) polyamines, 30 mM. Others were added in varying amounts, also as indicated in Table 7. When N,N'-dicyclohexylcarbodiimide (DCCD) was present, it was added 30 minutes prior to phage infection at a concentration of 0.1 mM. Polyamines, when used, were added at the time of infection. All other additives were present from the time when a 5% inoculum was added to fresh medium.

To determine the efficacy of the treatments, a phage titer was determined. Chloroform (1%) was added to the treated cultures 4 to 7 hours after infection, and samples were plated on lawns of *E. coli* B. This was designed as a screening procedure, so no attempt was made to remove unadsorbed phage.

Gel Analysis

To see what proteins were labeled with radioactive amino acids after infection, infected cells were pulse-labeled for 5 minutes with 1 μCi (^{14}C) of an amino acid mixture per ml of culture. The five-minute pulses were initiated at 1, 6, and 11 minutes after infection,

and terminated by the addition of 100 μ g of chloramphenicol per ml of medium and by chilling. The cells were then centrifuged, washed two times, and resuspended in 1/10 volume Laemmli electrophoresis buffer (58). The samples were then boiled for 5 minutes. Twenty microliters of each sample was loaded onto a 15% acrylamide slab gel and electrophoresed for 14 hours at 75 volts. The electrode buffer consisted of 0.192 M glycine and 0.1% SDS in 0.025 M Tris, pH 8.3. The dried gel was autoradiographed by exposing it to Kodak XRI film for 10 days.

The gel was prepared by layering a stacking gel over a previously solidified 15% running gel. A stock acrylamide solution contained 30% acrylamide, 0.8% bisacrylamide. The recipe for the stacking gel was as follows: 1.0 ml of the stock acrylamide solution, 2.5 ml of Tris-HCl (0.5 M, pH 6.8), 6.4 ml of distilled water, 0.1 ml of 10% SDS, 0.04 ml of 20% ammonium persulfate (freshly made), and 0.02 ml of N,N,N',N'-tetra-methyl-ethylenediamine (TEMED). The running gel consisted of the following: 20 ml of stock acrylamide solution, 10 ml of Tris-HCl (1.5 M, pH 8.8), 9.6 ml of distilled water, 0.4 ml of 10% SDS, 0.035 ml of 20% ammonium persulfate (freshly made), 0.01 ml of TEMED. Each type of gel was made by first mixing the acrylamide solution, Tris-HCl, distilled water, and ammonium persulfate. These ingredients were placed in a vacuum flask and held under vacuum for 3 to 5 minutes to degas thoroughly. Thereafter, the SDS and TEMED were added, the solution was swirled gently, and the gel was poured immediately.

Statistical Methods

The means of two sets of data were compared using Student's t-test, according to the method of Snedecor and Cochran (96). When

an equal number of data points were included in each set, t values were calculated using the formula: $t = (\bar{y}_1 - \bar{y}_2) \sqrt{n / (S_1^2 + S_2^2)}$, where \bar{y}_1 = the mean of data points in set 1, n = the number of data points in each set, S_1 = the standard deviation of the mean for data collected in set 1. P values were derived from a standard table. Application of the t -test presupposes that the data fit a normal t distribution.

Materials

^{42}K and the $[^{14}\text{C}]$ -labeled amino acid mixture were purchased from New England Nuclear, Boston; $[^{14}\text{C}]$ αMG was purchased from Amersham, Arlington Heights, Illinois. All other radiolabeled products were obtained from Schwarz/Mann, Orangeburg, New York.

NPN was the gift of W. A. Cramer of West Lafayette, Indiana. DCCD was purchased from Sigma Chemical Company, St. Louis, Missouri. All other chemicals used were analytical reagent grade, and are readily available from many producers.

RESULTS

Macromolecular Synthesis in T5 and T5h12⁻ Infections of RM 42, RM 43, and RM 39

It has been previously reported that transcription and translation cease at 6 to 10 minutes after T5 infection of ColIb⁺ *E. coli* (79). To determine when these changes occurred in our system, I measured the cumulative incorporation of an RNA and a protein precursor into acid-insoluble macromolecules at various times after infection. Uridine was used as an indicator of RNA synthesis, while tyrosine or proline was used to monitor protein synthesis. The cells I have used are described in Table 1, as are the infecting phage.

Typical results of experiments measuring incorporation of [³H]-uridine can be seen in Figure 1. It can be seen that RNA synthesis, measured in this way, continued for at least 30 minutes in productive infections, albeit at a slower rate than in uninfected cells (Fig. 1a). In infections of RM 43 (ColIb), however, uridine incorporation stopped at some time approximately 9 to 12 minutes after initiation of infections (Fig. 1b). Protein synthesis, as indicated by tyrosine or proline incorporation (Fig. 2 and Fig. 3) also stopped about 9 to 12 minutes after infection of ColIb-containing cells. RNA and protein synthesis proceeded at least 30 minutes in T5 wild-type infections of RM 39 (ColIb⁻) (Figs. 1c and 2c), and protein synthesis continued for at least 30 minutes in T5h12⁻ infections of RM 43 (ColIb) (Fig. 3b).

Figure 1. Uridine Incorporation into Acid-insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. [^3H] Uridine (1 μCi per ml, 5 μCi per μmole) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% TCA. Acid-insoluble material was collected on glass fiber filters, and the filters were washed. The amount of incorporated radioactivity was determined by liquid-scintillation counting. In Fig. 1a, the bacteria used were RM 42, which contain no plasmid. Fig. 1b represents results obtained with RM 43 (ColIb), and in Fig. 1c, RM 39 (ColIb h^-) was used.

●, uninfected cells
○, T5-infected cells

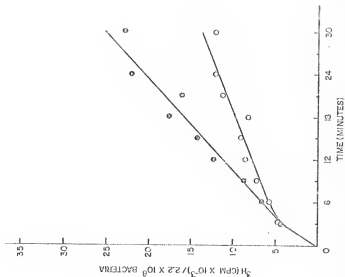


Figure 1a

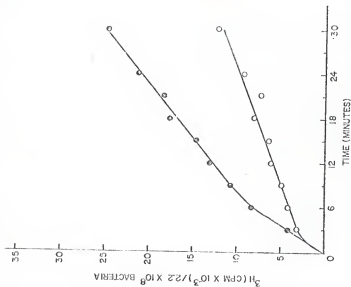


Figure 1c

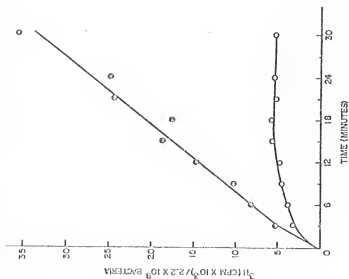


Figure 1b

Figure 2. Tyrosine Incorporation into Acid-insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. [^3H] Tyrosine ($1 \mu\text{Ci}$ per ml, $7.2 \mu\text{Ci}$ per μmole) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold $50\% \text{ TCA}$. Acid-insoluble material was recovered on glass fiber filters, and the amount of incorporated radioactivity was determined. In Fig. 2a, the host was RM 42; in Fig. 2b, the host was RM 43 (Collb); in Fig. 2c, the host was RM 39 (Collb h $^{-}$).

●, uninfected cells
○, T5-infected cells

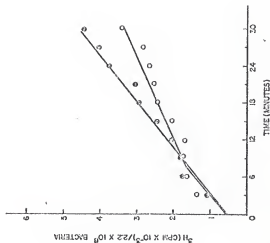


Figure 2a

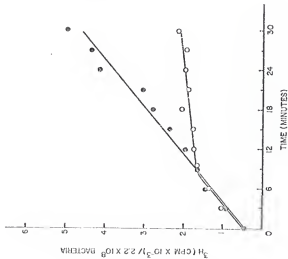


Figure 2b

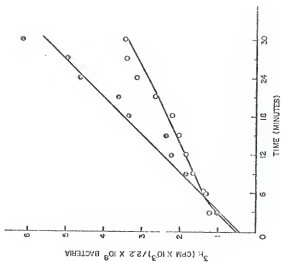


Figure 2c

Figure 3. Proline Incorporation into Acid-Insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39.

Cells were grown in synthetic medium. [^3H] Proline (1 μCi per ml, 4.8 μCi per μmole) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% TCA. Acid-insoluble material was recovered on glass fiber filters, and the amount of incorporated radioactivity was determined. In Fig. 3a, the host was RM 42; in Fig. 3b, the host was RM 43 (Colib h^-).

●, uninfected cells
○, T5-infected cells
▲, T5h12 $^-$ -infected cells

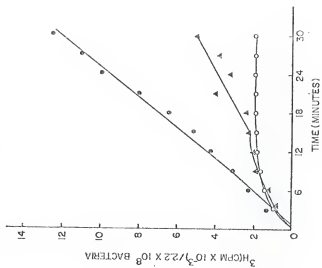


Figure 3b

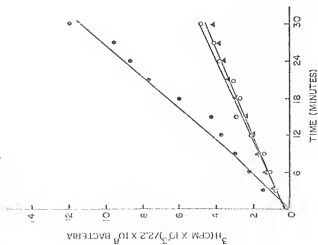


Figure 3a

Confirmation that RM 39 Contains Plasmid DNA

RM 39 is a strain sent to our laboratory by Richard Moyer and is said to contain a mutant ColIb factor which will allow T5 to replicate. I have used this strain to eliminate the likelihood that pathophysiological alterations observed during T5 infection of RM 43 are due to ColIb-borne genetic determinants unrelated to the determinants necessary for abortive infection. There is a possibility, however, that our laboratory stock of what is identified as RM 39 no longer contains the plasmid. This would explain T5's ability to replicate in the strain, but would invalidate the interpretations of experiments involving RM 39.

As can be seen in Table 1, RM 39 does indeed produce a substance, thought to be colicin Ib, which inhibits growth of bacteria sensitive to colicin Ib. This is presumptive evidence that the strain contains the ColIb factor. Since the presence of colicin Ib has not been rigorously demonstrated, however, I thought it wise to demonstrate the plasmid on an ethidium bromide-cesium chloride gradient.

Labeled DNA was extracted from RM 42, RM 43, and RM 39. [^{14}C]-Labeled DNA from RM 42 was mixed with [^3H]-labeled DNA from RM 43. As can be seen in Figure 4a, DNA from RM 43 appeared in two bands, one in the position characteristic of covalently closed, circular DNA (plasmid DNA), the other in the position characteristic of nicked circular DNA (*E. coli* chromosome) (21). DNA extracted from RM 42, however, banded in a single position characteristic of the *E. coli* chromosome. A second tube, which was centrifuged concurrently, contained [^3H]-labeled DNA from RM 43 and [^{14}C]-labeled DNA from RM 39

Figure 4. Ethidium Bromide-Cesium Chloride Gradients of ONA Extracted from RM 42, RM 43, and RM 39.

ONA in RM 43 was labeled by growing in the presence of [^3H] thymidine (1 μCi per ml, 1 μCi per 4 μg of thymidine), whereas DNA in RM 42 and RM 39 was labeled with [^{14}C] thymidine (1 μCi per ml, 1 μCi per 4 μg of thymidine). The ONA was extracted from the bacteria, and ONA from RM 42 and RM 43 was mixed in one tube, DNA from RM 39 and RM 43 was mixed in another tube. To the latter tube was added 0.1 μg of [^{32}P] labeled ONA of SV40. DNA forms 1 and 2 were banded by dye-buoyant density equilibrium centrifugation. Fractions of about 0.25 ml were removed and mixed with TCA (10%-final concentration). From each sample, 0.15 ml was filtered over glass fiber filters and washed. The number of ^3H , ^{14}C , and ^{32}P counts on each filter were determined by liquid-scintillation counting. These figures are plotted. Fig. 4a represents the RM 42 - RM 43 mixture, whereas Fig. 4b represents the RM 42 - RM 43 - SV40 mixture.

●, RM 42 DNA (^{14}C)
 ○, RM 43 ONA (^3H)
 ▲, RM 39 ONA (^{14}C)
 ■, SV40 ONA (^{32}P)

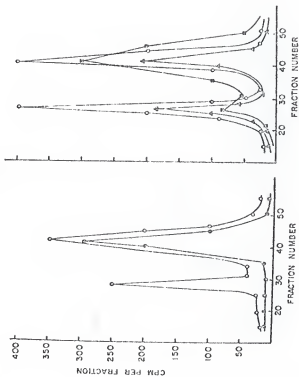


Figure 4a

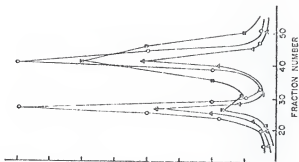


Figure 4b

(Fig. 4b). Additionally, this tube contained [^{32}P]-labeled SV40 DNA which is known to consist of both covalently closed, circular DNA and nicked, circular DNA (4). As before, two peaks appeared, each containing [^3H] counts of RM 43 DNA. These peaks were in positions identical to peaks containing [^{32}P] counts of SV40 DNA and [^{14}C] counts of RM 39 DNA. The results conclusively show that both RM 39 and RM 43 contain plasmid DNA.

Confirmation that T5h12⁻ Infections Begin Promptly

Studies using T5h12⁻ mutants have been performed to eliminate the likelihood that pathophysiological alterations observed during T5 infections of RM 43 are due to phage genetic determinants unrelated to the A3 gene function. If the T5h12⁻ phage were very slow to initiate infections in RM 43, normal bacterial physiological processes would be expected to continue in the T5h12⁻-RM 43 suspension for some time. This could invalidate interpretations of the experiments using these mutants.

To confirm that T5h12⁻ phage adsorb and initiate infections promptly, breakdown of host DNA, an event normally occurring in the first minutes of infection, was monitored. As can be seen in Figure 5, breakdown of prelabeled host DNA into acid-soluble fragments occurs promptly after T5 wild-type or T5h12⁻ infection of RM 42, RM 43, or RM 39. From these results, it is evident that T5h12⁻ phage are able to normally adsorb to and initiate infection in RM 43.

Amino Acid Accumulation in T5 and T5h12⁻ Infections of RM 42, RM 43, and RM 39

In an attempt to clarify the relationship between the inhibition of RNA and protein synthesis and the membrane changes previously observed

Figure 5. Host DNA Breakdown After T5h12⁻ Infection of RM 42, RM 43, and RM 39.

DNA was labeled by growing the cells in the presence of [³H] thymidine (0.4 μ Ci per ml, 1 μ Ci per 50 μ g of thymidine). The cells were spun, concentrated, infected, and rediluted in medium without radiolabeled thymidine. At times thereafter, 0.9 ml samples were removed and placed in 0.1 ml of 50% TCA. Of this, 0.4 ml was filtered over glass fiber filters and washed. The number of acid-precipitable counts were determined by liquid-scintillation counting. In Fig. 5a, the host is RM 42; in Fig. 5b, the host is RM 43 (Col1b), whereas RM 39 (Col1b⁻) is the host in Fig. 5c.

- , uninfected cells
- , T5-infected cells
- ▲, T5h12⁻-infected cells

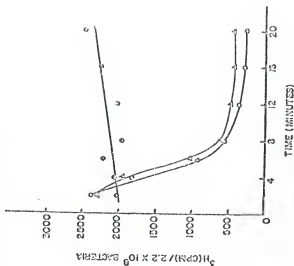


Figure 5a

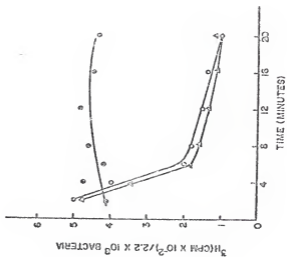


Figure 5c

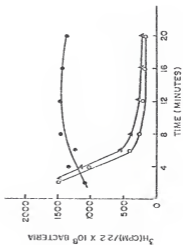


Figure 5b

(19), and to more specifically define the membrane defects, I looked at accumulation of two amino acids. The transport systems of proline and glutamine are fairly well characterized (6,7), and both systems require an "energized" membrane. Proline requires membrane-bound transport proteins (still present when purified membrane vesicles are made), but does not require a high-energy phosphorylated intermediate. Glutamine, on the other hand, relies upon periplasmic binding proteins and needs ATP to energize its active transport. I wanted to see when, if at all, these uptake systems were inhibited during abortive infection. If they were inhibited, I wanted to know if they remained functional during infections involving h^- mutants of the phage or the plasmid.

In these experiments, aliquots of cells were removed at various times after infection and incubated with chloramphenicol for one minute -- a time sufficient to totally stop protein synthesis -- prior to adding the labeled amino acid. The results, therefore, reflect only net uptake of the amino acid, and are not indicative of incorporation into proteins.

When wild-type T5 infected any of our three bacterial strains, there was a slight drop in proline accumulation at 5 minutes after infection (Fig. 6a and Table 2). By 10 minutes into the infectious process, however, net uptake returned to uninfected levels in all cases, and continued to increase in both infected RM 42 and infected RM 39 (ColIb h^-). In abortively infected RM 43 (ColIb), however, there was a drastic reduction in the ability to accumulate proline between 10 and 15 minutes. If T5h12 $^-$ phage infected RM 43 (ColIb)

Figure 6. Proline Accumulation in 60 Seconds by Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. At the indicated times after infection, samples were removed and mixed with [^3H] proline (1 μCi per ml, 25 μCi per μmole). Aliquots were removed at 30 seconds and 60 seconds thereafter and filtered through glass fiber filters. Here, the amount of radioactive proline accumulated in 60 seconds by infected cells is expressed as a % of the amount taken up by uninfected controls in the same period of time. Fig. 6a represents T5wt infections of *E. coli* RM 42, RM 43 (Colib), and RM 39 (Colib h). Fig. 6b represents T5h12 $^-$ infections of the same three bacterial strains.

●, RM 42
○, RM 43 (Colib)
▲, RM 39 (Colib h)

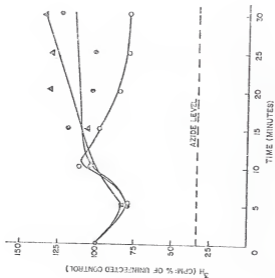


Figure 6b

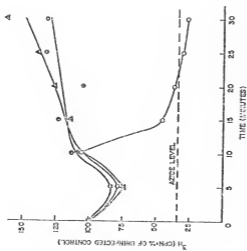


Figure 6a

TABLE 2. Proline Accumulation (in 60 seconds) by Infected RM 42, RM 43, and RM 39

TIME AFTER PHAGE	RM 42-T5wt vs RM 43-T5wt (3)	RM 43-T5wt vs RM 39-T5wt (3)
5 minutes	83 ± 2	78 ± 7
10 minutes	113 ± 15	106 ± 24
15 minutes	123 ± 27 *	46 ± 7 *
20 minutes	105 ± 19 *	37 ± 6 *
25 minutes	133 ± 32 *	30 ± 7 *
30 minutes	132 ± 51 *	27 ± 5 *
NaN ₃ + uninfected cells	12 ± 8	14 ± 4

TIME AFTER PHAGE	RM 43-T5h12 ⁻ vs RM 43-T5wt (3)	RM 42-T5h12 ⁻ vs RM 43-T5h12 ⁻ vs RM 39-T5h12 ⁻ (4)
5 minutes	79 ± 10	79 ± 16
10 minutes	96 ± 6	104 ± 26
15 minutes	92 ± 16 *	119 ± 31
20 minutes	83 ± 12 *	103 ± 25
25 minutes	85 ± 9 *	102 ± 25
30 minutes	83 ± 9 *	124 ± 26 *
NaN ₃ + uninfected cells	32 ± 2	32 ± 23

() indicates a number of experiments done in each group

* indicates a difference between the 2 samples with a confidence level, $p < 0.05$ [³H] Proline accumulation by infected cells was determined as outlined in MATERIALS AND METHODS. The results represent %'s of uptake, relative to that of uninfected control samples, in 60 seconds.

(Fig. 6b), there was only a slight reduction in net uptake as compared to that seen during infections of RM 42 or RM 39 (ColIb^h). In comparing results observed during T5h12⁻ infections of RM 42 and RM 43 (ColIb) by Student's t-test, only the difference in values at 30 minutes was significant. This slight loss in net uptake in colicinogenic hosts was perhaps due to the 50% plating efficiency of the mutant phage on RM 43 (ColIb). When T5 wild-type and T5h12⁻ infections of RM 43 (ColIb) were compared directly, statistically significant differences were observed at all times after 10 minutes. In summary, the ability to accumulate proline was drastically reduced between 10 and 15 minutes in nonpermissive hosts. This inhibition was not observed when h mutations on either the phage or plasmid allowed productive infection to occur.

Similar results are seen when glutamine uptake is measured (Fig. 7 and Table 3). After observing a reduction in uptake ability at five minutes after T5 wild-type infections of any one of the three strains, we saw a gradual recovery in uptake ability during productive infections. When RM 43 (ColIb) was the host, uptake ability fell progressively, and there was a statistically significant difference, as compared to infections of RM 42, by 15 minutes. Although the 10 minute values were not significantly different, in some experiments there was indeed a large disparity. The mean 10-minute value of glutamine uptake in infected RM 43 (ColIb) was lower than the value of infected RM 39 (ColIb^h). Infection of RM 43 (ColIb) with T5h12⁻ or T5 wild-type showed that the h mutation prevented the fall in glutamine uptake. The cell's ability to accumulate glutamine, then, as was the

Figure 7. Glutamine Accumulation in 90 Seconds by Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. At the indicated times, samples were removed and mixed with [^{14}C] glutamine (0.5 μCi per ml, 5 μCi per μmole). At 30, 60, and 90 seconds thereafter, aliquots were removed and filtered through glass fiber filters. The results here are for the 90-second pulses, expressed as a % of the net amount taken up by uninfected controls in the same period of time. Fig. 7a represents T5wt infections of RM 42, RM 43 (ColIb), and RM 39 (ColIb h^-). Fig. 7b represents T5h12 $^-$ infections of the same three strains.

●, RM 42
○, RM 43 (ColIb)
▲, RM 39 (ColIb h^-)

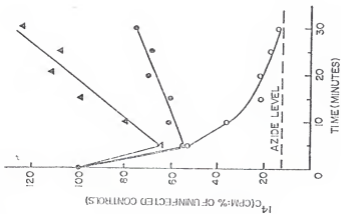


Figure 7a

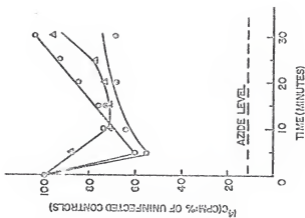


Figure 7b

TABLE 3. Glutamine Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39

TIME AFTER PHASE	RM 42-T5wt vs RM 43-T5wt (4)	RM 43-T5wt vs RM 39-T5wt (3)
5 minutes	54 ± 25	50 ± 11
10 minutes	61 ± 33	35 ± 13
15 minutes	60 ± 30	21 ± 11
20 minutes	69 ± 31	18 ± 10
25 minutes	68 ± 45	17 ± 10
30 minutes	75 ± 48	16 ± 7
NaI ₃ + uninfected cells	17 ± 17	15 ± 3

TIME AFTER PHASE	RM 43-T5h12 ⁻ vs RM 43-T5wt (4)	RM 42-T5h12 ⁻ vs RM 43-T5h12 ⁻ vs RM 39-T5h12 ⁻ (1)
5 minutes	65 ± 14	56 ± 3
10 minutes	66 ± 21	38 ± 16
15 minutes	75 ± 16	24 ± 12
20 minutes	71 ± 19	25 ± 15
25 minutes	73 ± 28	18 ± 10
30 minutes	74 ± 28	16 ± 8
NaI ₃ + uninfected cells	12 ± 5	12 ± 5

() indicates the number of experiments done in each group

* indicates a difference between the 2 samples with confidence level, $p < 0.05$ [¹⁴C] Glutamine accumulation by infected cells was determined as outlined in MATERIALS AND METHODS. The results represent %s of uptake, relative to that of uninfected control samples, in 90 seconds.

case with proline uptake, was drastically reduced during abortive infection. This dysfunction was prevented by phage or plasmid mutations which also allow productive infection to proceed. There was a tendency for glutamine uptake to decline a bit earlier than proline uptake but the two uptake systems were not compared in the same experiment.

Confirmation that Inhibited Proline and Glutamine Accumulation is Not Due Solely to Inhibited Protein Synthesis

One possibility is that the decreased net amino acid uptake is due solely to cessation of protein synthesis. There could, for example, be an accumulation of amino acids in T5-infected RM 43 after protein synthesis stops at 9 to 12 minutes after infection. This might limit net uptake of proline or glutamine from the medium.

I therefore treated uninfected or T5-infected cells with chloramphenicol 1 minute or 15 minutes prior to determining net uptake of proline or glutamine from the medium. Even 15 minutes after inhibition of protein synthesis with the antibiotic, there was no decrease in accumulation of either amino acid from the medium when glucose was the carbon source (Table 4). These results eliminate the likelihood that the decrease in net uptake of amino acids during abortive infection is due only to cessation of protein synthesis.

α -Methylglucoside Accumulation in T5 and T5h12⁻
Infection of RM 42, RM 43, and RM 39

Since accumulation of either proline or glutamine requires an energized membrane, I decided to measure net uptake of a substance which is transported via a group translocation reaction, and does not require membrane polarization. α MG is a nonmetabolizable analogue of glucose taken into the cell using the phosphotransferase system

TABLE 4. Proline and Glutamine Accumulation in Uninfected and Infected RM 43 After 1 Minute or 15 Minutes of Incubation with Chloramphenicol

	Proline (3)		Glutamine (3)	
	Uninfected Cells	5 Minutes After T5	Uninfected Cells	5 Minutes After T5
Chloramphenicol for 1 Minute	970 \pm 166	875 \pm 52	1331 \pm 98	793 \pm 94
Chloramphenicol for 15 Minutes	940 \pm 198	1033 \pm 190	1719 \pm 49	1047 \pm 118

() indicates the number of experiments done in each group

[³H] Proline or [¹⁴C] glutamine accumulation was determined as outlined in MATERIALS AND METHODS, except that, where indicated, chloramphenicol was allowed to inhibit protein synthesis for 15 minutes prior to measuring accumulation of the labeled amino acid. Results represent raw counts accumulated by approximately 2.2 x 10⁸ cells in 60 seconds (proline) or in 90 seconds (glutamine).

and energy derived from phosphoenolpyruvate (57). I wanted to see if this transport system was functional in abortively infected cells.

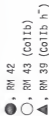
Phage infection, whether productive or abortive, increased rather than decreased α MG accumulation (Fig. 8 and Table 5). By 5 minutes after infection, however, the increase was much greater in the abortive infection than it was in the productive infection. As was seen for proline or glutamine uptake, the absence of the normal phage or plasmid h gene product eliminated the effect. The augmented accumulation during abortive infection was inhibited by NaF, as is normal for transport systems energized by phosphoenolpyruvate (57), showing that the increased uptake was via the phosphotransferase system and not a new uptake system. After 10 minutes of infection in a non-permissive host, the increase in α MG accumulation began to decline, although the total uptake was still much greater than that during productive infection.

Glucose Incorporation into Macromolecules in T5 and T5h12- Infection of RM 42, RM 43, and RM 39

The rate of protein synthesis or nucleic acid synthesis measured by incorporation of radiolabeled precursors depends first upon the cells' ability to take up the precursor from the medium. Since the uptake of amino acids into the soluble pools of T5-infected cells was severely depressed during abortive infection, it cannot be said that protein synthesis was necessarily inhibited. Since the glucose transport system appeared to remain functional, as measured by α MG uptake, we have looked for the incorporation of label from [14 C]-glucose into acid-insoluble material (Fig. 9).

Figure 8. α -Methylglucoside Accumulation in 90 Seconds by Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. At the indicated times after infection, samples were removed, spun, and washed with M9. After a second wash, the cells were resuspended in M9 with 18 μ g of glucose per ml, and 0.1 ml of [14 C] α MG (10 μ Ci per ml, 184 μ Ci per μ mole) was added to 0.9 ml of the cell suspension. Aliquots were removed at 45 and 90 seconds, thereafter, and were filtered through glass fiber filters. The results here are for the 90-second pulses, expressed as a % of the amount taken up by uninfected controls in the same period of time. In Fig. 8a, the infecting phage is T5wt, whereas in Fig. 8b, it is T5h12⁻.



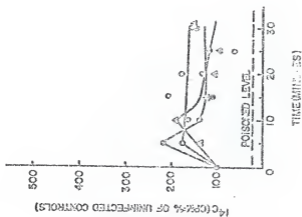


Figure 8b

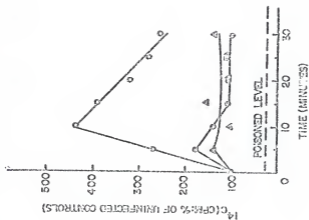


Figure 8a

TABLE 5. α -Methylglucoside Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39

TIME AFTER PHASE	RM 42-T5wt vs RM 43-T5wt	(4)	RM 43-T5wt vs RM 39-T5wt	(3)
5 minutes	177 \pm 40	*	254 \pm 18	*
10 minutes	140 \pm 12	*	439 \pm 74	*
15 minutes	108 \pm 19	*	392 \pm 85	*
20 minutes	107 \pm 20	*	323 \pm 9	*
25 minutes	114 \pm 18	*	282 \pm 48	*
30 minutes	100 \pm 28	*	257 \pm 30	*
NaCl and NaF + uninfected cells	25 \pm 7		25 \pm 10	
			18 \pm 7	
TIME AFTER PHASE	RM 43-T5h12 ⁻ vs RM 43-T5wt	(1)	RM 42-T5h12 ⁻ vs RM 43-T5h12 ⁻	vs RM 39-T5h12 ⁻ (2)
5 minutes	202	245	219 \pm 6	177 \pm 59
10 minutes	225	354	137 \pm 19	167 \pm 17
15 minutes	169	313	112 \pm 14	211 \pm 130
20 minutes	164	313	134 \pm 24	180 \pm 36
25 minutes	153	228	118 \pm 4	65 \pm 13
30 minutes	134	260	148 \pm 15	157 \pm 61
NaCl and NaF + uninfected cells	20	37	18 \pm 8	25 \pm 6
				17 \pm 1

() indicates the number of experiments done in each group

* indicates a difference between the 2 samples with a confidence level, $p < 0.05$ [¹⁴C] α MG accumulation by infected cells was determined as outlined in MATERIALS AND METHODS. The results represent %'s of uptake, relative to that of uninfected control samples, in 90 seconds.

Figure 9. Glucose Incorporation into Acid-Insoluble Macromolecules in Uninfected and Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. [^{14}C] Glucose (0.5 μCi per ml, 0.1 μCi per mole) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% TCA. Acid-insoluble material was collected on glass fiber filters and the filters washed. The amount of incorporated radioactivity was determined by liquid scintillation counting. In Fig. 9a, the bacteria used were RM 42; in Fig. 9b, the host was RM 43 (ColIb); in Fig. 9c, the host cells were RM 39 (ColIb h^-).

●, uninfected cells
○, T5-infected cells
▲, T5h12⁻-infected cells

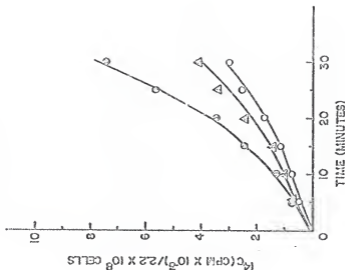


Figure 9a

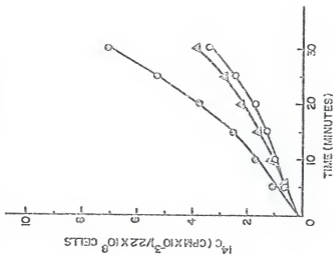


Figure 9c

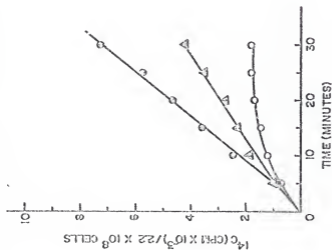


Figure 9b

Cumulative incorporation of glucose into an insoluble form was inhibited in the abortively infected RM 43 (ColIb) at about 10 minutes after infection (Fig. 9b). This was not the case during T5 infections of RM 42 or RM 39 (ColIb⁻) (Fig. 9a and 9c), nor was it true of T5h12⁻ infections of RM 43 (ColIb) (Fig. 9b). Since the glucose transport system remained operative for at least 30 minutes during abortive infection, it appeared that macromolecular synthesis per se was inhibited at about 10 minutes. This conclusion must still be regarded as tentative, however, since the results could be explained by leakage of intermediary metabolites of glucose. Further investigation of this problem may be warranted.

Fluorescence Intensity of NPN During
T5 and T5h12⁻ Infection of RM 42, RM 43, and RM 39

The results of the uptake studies suggested that membrane depolarization was occurring at about 10-12 minutes after T5 infection of ColIb-containing hosts. If true, when cells are infected in the presence of NPN, the fluorescence intensity emitted should increase at about this time, since chemicals known to depolarize the bacterial membrane have this effect (44).

As can be seen in Fig. 10, when T5 infects any of the 3 strains in the presence of NPN, there is an increase in fluorescence intensity at about 3 minutes after infection and another at about 6 minutes after infection. This biphasic effect has been observed previously (41) and is thought to reflect changes in the cell membrane at the times of first- and second-step DNA transfer. Data presented below are consistent with this interpretation.

Figure 10. Fluorescence Intensity of N-phenyl-1-naphthylamine during T5 and T5h12⁻ Infections of RM 42, RM 43, and RM 39.

Cells were grown, concentrated, and infected in synthetic medium. At time zero, the infected cells were put into prewarmed growth medium containing the fluorescent probe. Relative fluorescence intensity was recorded in arbitrary units using a scanning fluorimeter. In Fig. 10a, the infecting phage was T5wt; in Fig. 10b, the phage was T5h12⁻.

●, RM 42

○, RM 43 (Col1b)

▲, RM 39 (Col1b h⁻)

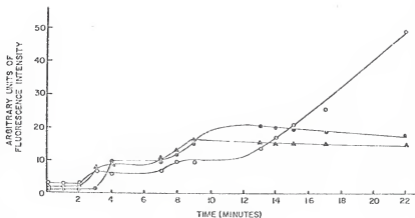


Figure 10a

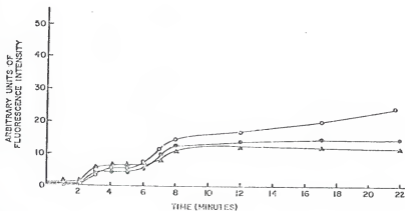


Figure 10b

Only in T5-infected RM 43 (Col1b) is a third rise noted. The final increase in the fluorescence signal begins about 12 minutes after infection of the colicinogenic hosts, coinciding with the time when macromolecular synthesis ceases and uptake systems fail. Only the first two rises are seen after infection of RM 42 or RM 43 (Col1b) by the T5 host range mutant, T5h12⁻. The latter finding is consistent with the idea that the third rise in fluorescence yield is related to the abortive process.

Potassium Efflux During T5 Infection of RM 42, RM 43, and RM 39

The third increase in fluorescence intensity during abortive infection suggests that membrane depolarization occurs. To collect further evidence of this, I decided to measure efflux of potassium ions during T5 infection.

After preloading cells with ⁴²K, a portion was infected with T5, whereas another portion remained uninfected. Samples of each were taken at intervals. As can be seen in Table 6 and Figure 11, labeled potassium ions pass to the medium during the first 4 to 8 minutes of productive or abortive infection. Thereafter, however, efflux stops in infected RM 42 or RM 39. Efflux continues in the abortive infection, though, so that by 10 minutes after infection there is a marked difference between the cells which are productively or abortively infected as regards labeled potassium content.

Attempts to Prevent Abortion of T5 Infection of Col1b-Containing Cells

In several other systems where abrupt cessation of energy-requiring events occurs in conjunction with membrane alterations,

Figure 11. Potassium Efflux from T5-Infected RM 42, RM 43, and RM 39.

Cells were grown in the presence of radio-labeled potassium (0.1 μ Ci per ml, 0.18 μ Ci per mg of K), spun, washed, and infected or mock-infected. At time zero, the infected or mock-infected cells were resuspended in medium without the radiolabel. At the indicated intervals thereafter, 0.9 ml samples were removed, filtered over glass fiber filters, and the filters were washed. The amount of residual, intracellular labeled potassium was determined by liquid scintillation counting. The results represent the amount of ^{42}K remaining inside infected cells, expressed as a % of the amount remaining in mock-infected cells taken at the same time point.

●, RM 42

○, RM 43 (Col1b)

▲, RM 39 (Col1b h^-)

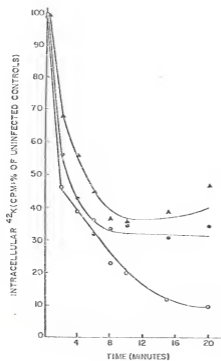


Figure 11

TABLE 6. Potassium Efflux From T5-Infected
RM 42, RM 43, and RM 39

Minutes After Infection	RM 42 (3)	RM 43 (3)	RM 39 (1)
2	56 \pm 2	46 \pm 27	68
4	43 \pm 10	39 \pm 28	56
6	32 \pm 7	36 \pm 26	45
8	34 \pm 8	23 \pm 12	37
10	*35 \pm 7	*20 \pm 8	36
15	*31 \pm 9	*12 \pm 9	39
20	*35 \pm 11	*10 \pm 3	47

() indicates the number of experiments done in each group

* indicates a statistically significant difference, $p < 0.05$, between mean values for infected RM 42 and infected RM 43. Since efflux from infected RM 39 was only measured in one experiment, statistical analysis was not used to compare these results with those obtained with infected RM 42 and RM 43.

RM 42, RM 43, and RM 39 were preloaded with ^{42}K . At intervals, uninfected and infected cells were taken to determine the amount of potassium efflux. The results represent the amount of ^{42}K remaining inside infected cells, expressed as a % of the amount remaining in uninfected cells taken at the same time point.

means have been devised to prevent interruption of normal processes. I decided to screen a variety of these treatments to determine if any would be effective in allowing T5 to replicate in Col1b-containing cells.

DCCD is an inhibitor of membrane-bound ATP'ase (43), and has been used effectively in conjunction with high concentrations of magnesium and potassium to prevent colicin K-mediated cell death (56). Sucrose or polyamines, or high concentrations of either magnesium or calcium with low sodium concentrations have been effective in preventing abortion of T4-II mutant infections of E. coli k-12 (λ) (14).

Table 7 outlines my results. In no case was a treatment effective in circumventing abortive T5 infection, as determined by titering phage present 4 to 7 hours after infection. Since calcium is required for successful T5 replication (59, 77, 78), high calcium concentrations were also tested. These trials were similarly unsuccessful.

Macromolecular Synthesis in T5 Wild-Type
and T5A1⁻ Infection of RM 42, RM 43, and RM 39

It was my opinion that a previous report (45) suggesting that early protein synthesis was necessary for abortive T5 infection was inconclusive. Therefore, I used mutants of T5 to restudy this question. The T5A1⁻ phage mutants are eminently suited for this purpose. As discussed in the INTRODUCTION, these mutants cannot produce any class II or class III proteins, since the A1 function is required for second-step DNA transfer. A1⁻ mutants are also unable to shut off class I gene expression, another function controlled by the A1 gene (71). I have used two mutants, T5am16d and T5amH27, each of which has an amber mutation in the A1 region (5).

TABLE 7. Attempts to Prevent Abortion of T5 Infection of Colib⁺ Hosts

Bacteria	Potassium	Calcium	Magnesium	Sodium	Sucrose	DCCO	Polyamine	Titer
RM 42	-	0.5 mM	-	+	-	-	-	1 x 10 ¹¹ /ml
RM 42	-	0.5 mM	-	+	-	+	-	3 x 10 ¹⁰ /ml
RM 43	-	0.5 mM	-	+	-	-	-	6 x 10 ⁹ /ml
RM 43	-	0.5 mM	-	+	-	+	-	1 x 10 ⁸ /ml
RM 43	-	0.5 mM	+	-	-	+	-	1 x 10 ⁸ /ml
RM 43	+	0.5 mM	+	-	-	-	-	1 x 10 ⁸ /ml
RM 43	+	0.5 mM	+	-	-	+	-	1 x 10 ⁹ /ml
RM 43	-	0.5 mM	-	-	+	-	-	6 x 10 ⁸ /ml
RM 43	-	0.5 mM	+	-	+	-	-	6 x 10 ⁸ /ml
RM 43	+	0.5 mM	+	-	+	-	-	2 x 10 ⁸ /ml
RM 43	-	0.5 mM	-	+	-	-	-	7 x 10 ⁸ /ml
RM 43	-	0.5 mM	+	-	-	-	cadaverine	1 x 10 ⁹ /ml
RM 43	-	0.5 mM	-	+	-	-	cadaverine	6 x 10 ⁸ /ml
RM 43	-	0.5 mM	+	-	-	-	arginine	1 x 10 ⁹ /ml
RM 43	-	0.5 mM	-	+	-	-	arginine	1 x 10 ⁸ /ml
RM 43	-	0.5 mM	+	-	-	-	spermidine	3 x 10 ⁸ /ml
RM 43	-	0.5 mM	+	-	-	-	spermidine	3 x 10 ⁸ /ml
RM 43	-	4 mM	+	-	-	-	-	6 x 10 ⁸ /ml
RM 43	-	4 mM	+	-	+	-	-	1 x 10 ⁸ /ml
RM 43	-	4 mM	+	-	+	+	-	1 x 10 ⁸ /ml
RM 43	-	4 mM	+	-	+	+	-	1 x 10 ⁸ /ml

Cells were grown and infected as outlined in MATERIALS AND METHODS.

Where indicated, additives were present at concentrations also indicated in MATERIALS AND METHODS. At 4 to 7 hours after infection, the phage were plated on a lawn of *E. coli* 8 to determine the titer.

Figure 12 represents cumulative [^3H] uridine incorporation into TCA-precipitable material in infected bacteria, while Figure 13 shows the results of the analogous experiments with [^3H] tyrosine. Uptake of the labeled precursors and incorporation into macromolecules continue for at least 24 minutes after RM 42 is infected with either wild-type T5 or with an Al^- mutant. In the corresponding infections of RM 43 (ColIb), however, incorporation of both uridine and tyrosine into macromolecules stops at 6 to 12 minutes after either mutant or wild-type phage is added. The possibility that this shut-off in RM 43 (ColIb) is due to a property of the colicinogenic host unrelated to abortive infection is ruled out by the fact that incorporation of both substances in infected RM 39 (ColIb h^-) continues for the duration of the experiments. From these experiments, it is evident that only class I (pre-early) gene products are necessary to induce the abortive cessation of RNA and protein synthesis.

Proline, Glutamine, and αMG Accumulation
in T5 Wild-type and T5 $\Delta\text{ml}6\text{d}$ -Infected RM 42, RM 43, and RM 39

Previously, it was shown that decreased ability of host cells to accumulate proline and glutamine is characteristic of abortive infections, while enhancement of net uptake of αMG occurs. Experiments comparing the ability of T5 $\Delta\text{ml}6\text{d}$ - or wild-type T5-infected RM 43 (ColIb) and RM 39 (ColIb h^-) to transport these three substances are shown (Fig. 14, 15, 16; Tables 8, 9, 10). The inhibition of proline and glutamine net uptake is the same whether a wild-type or an Al^- mutant infect a non-permissive host. Likewise, the stimulation of αMG transport occurs when either Al^- mutants or wild-type phage are used.

Figure 12. Uridine Incorporation into Acid-insoluble Macromolecules in Uninfected and T5A17-Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. [^3H] Uridine (1 μCi per ml, 5 μCi per μmole) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% TCA. Acid-insoluble material was collected on glass fiber filters, and the filters were washed. The amount of incorporated radioactivity was determined by liquid scintillation counting. In Fig. 12a, the bacteria used were RM 42; Fig. 12b represents results obtained with RM 43 (ColIb), and in Fig. 12c, RM 39 (ColIb $^-$) was used.

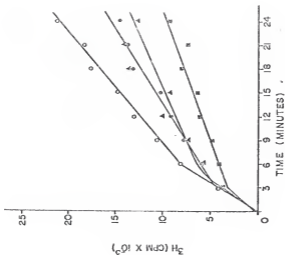


Figure 12a

- , control, no infection
- ◼, T5wt infection
- , T5am16d infection
- ▲, T5amH27 infection

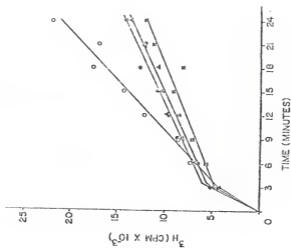


Figure 12c

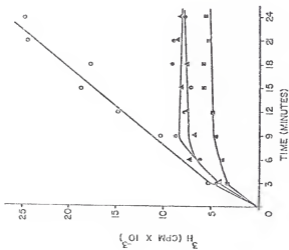


Figure 12b

Figure 13. Tyrosine Incorporation into Acid-insoluble Macromolecules in Uninfected and T5A1--Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. [^3H] Tyrosine (1 μCi per ml, 7.2 μCi per μmole) was added at time zero. Samples of 0.9 ml were removed at the indicated times and mixed with 0.1 ml of cold 50% TCA. Acid-insoluble material was recovered on glass fiber filters, and the amount of incorporated radioactivity was determined. In Fig. 13a, the host was RM 42; in Fig. 13b, the host was RM 43 (Col1b); in Fig. 13c, the host was RM 39 (Col1b h $^{-}$).

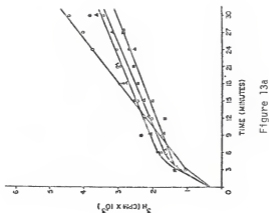


Figure 13a

- , control, no infection
- , T5wt infection
- , T5am16d infection
- ▲, T5amH27 infection

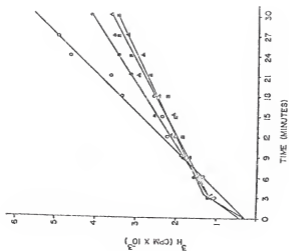


Figure 13b

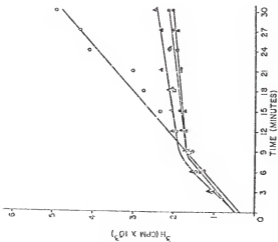


Figure 13c

Figure 14. Proline Accumulation in 60 Seconds by T5A1⁻-Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. At the indicated times after infection, samples were removed and mixed with [³H] proline (1 μ Ci per ml, 25 μ Ci per μ mole). Aliquots were removed at 30 seconds and 60 seconds thereafter and filtered through glass fiber filters. Here, the amount of radioactive proline accumulated in 60 seconds by infected cells is expressed as a % of the amount taken up by uninfected controls in the same period of time.

- , T5am16d-infected RM 42
- , T5am16d-infected RM 43 (Col1b)
- ▲, T5am16d-infected RM 39 (Col1b h⁻)
- , T5wt-infected RM 43 (Col1b)

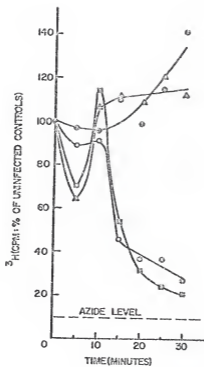


Figure 14

TABLE 8. Proline Accumulation (in 60 seconds) by Infected RM 42, RM 43, and RM 39

CONDITION	RM 42-T5am16d vs RM 43-T5am16d	RM 39-T5am16d vs RM 43-T5am16d	RM 43-T5am16d vs RM 43-T5wt(1)
5 minutes after phage	97 \pm 40	89 \pm 15	88
10 minutes after phage	96 \pm 24	91 \pm 4	87
15 minutes after phage	110 \pm 15	* 46 \pm 2	45
20 minutes after phage	99 \pm 8	* 37 \pm 13	40
25 minutes after phage	115 \pm 9	* 37 \pm 10	41
30 minutes after phage	142 \pm 26	* 27 \pm 9	32
NaN ₃ + uninfected cells	10 \pm 5	6 \pm 1	8

() indicates the number of experiments done in each group

* indicates a difference between the 2 samples with a confidence level, $p < 0.05$

[³H] Proline accumulation by infected cells was determined as outlined in MATERIALS AND METHODS. The results represent %'s of uptake, relative to that of uninfected control samples, in 60 seconds.

Figure 15. Glutamine Accumulation in 90 Seconds by T5A1⁻-Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. At the indicated times, samples were removed and mixed with [¹⁴C] glutamine (0.5 μ Ci per ml, 5 μ Ci per μ mole). At 30, 60, and 90 seconds thereafter, aliquots were removed and filtered through glass fiber filters. The results here are for the 90-second pulses, expressed as a % of the amount taken up by uninfected controls in the same period of time.

- , T5am16d-infected RM 42
- , T5am16d-infected RM 43 (Col1b)
- ▲, T5am16d-infected RM 39 (Col1b h⁻)
- , T5wt-infected RM 43 (Col1b)

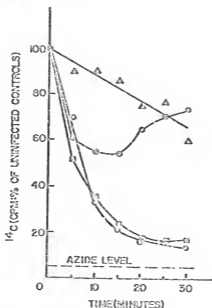


TABLE 9. Glutamine Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39

CONDITION	RM 42-T5am16d	vs RM 43-T5am16d	(3)	RM 43-T5am16d	vs RM 43-T5wt	(3)
5 minutes after phage	61 ± 17		70 ± 24	70 ± 24	52 ± 20	
10 minutes after phage	55 ± 12	*	33 ± 10	33 ± 10	35 ± 2	
15 minutes after phage	54 ± 11	*	21 ± 2	21 ± 2	24 ± 7	
20 minutes after phage	65 ± 24	*	16 ± 5	16 ± 5	18 ± 5	
25 minutes after phage	71 ± 31	*	16 ± 5	16 ± 5	17 ± 4	
30 minutes after phage	74 ± 36	*	14 ± 5	14 ± 5	17 ± 6	
NaN ₃ + uninfected cells	7 ± 2		5 ± 4	5 ± 4	-	

CONDITION	RM 39-T5am16d	vs RM 43-T5	16d	(2)
5 minutes after phage	90 ± 8		82 ± 15	
10 minutes after phage	90 ± 6	*	31 ± 13	
15 minutes after phage	86 ± 1	*	20 ± 2	
20 minutes after phage	75 ± 17	*	13 ± 2	
25 minutes after phage	77 ± 2	*	19 ± 0	
30 minutes after phage	60 ± 8	*	16 ± 5	
NaN ₃ + uninfected cells	5 ± 4		2 ± 2	

() indicates the number of experiments done in each group

* indicates a difference between the 2 samples with a confidence level, $p < 0.05$

[¹⁴C] Glutamine accumulation by infected cells was determined as outlined in MATERIALS AND METHODS. The results represent percentages of uptake, relative to that of uninfected control samples, in 90 seconds.

Figure 16. α -Methylglucoside Accumulation in 90 Seconds by T5A1--Infected RM 42, RM 43, and RM 39.

Cells were grown and infected in synthetic medium. At the indicated times after infection, samples were removed, spun, and washed with M9. After a second wash, the cells were resuspended in M9 with 18 μ g of glucose per ml, and 0.1 ml of [14 C] α MG (10 μ Ci per ml, 184 μ Ci per mole) was added to 0.9 ml of the cell suspension. Aliquots were removed at 45 and 90 seconds, thereafter, and were filtered through glass fiber filters. The results here are for the 90-second pulses, expressed as a % of the amount taken up by uninfected controls in the same period of time.

- , T5am16d-infected RM 42
- , T5am16d-infected RM 43 (Col1b)
- ▲, T5am16d-infected RM 39 (Col1b h⁻)
- , T5wt-infected RM 43 (Col1b)

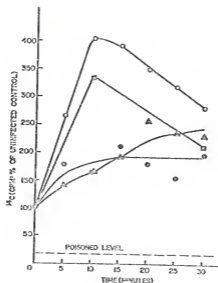


TABLE 10. α -Methylglucoside Accumulation (in 90 seconds) by Infected RM 42, RM 43, and RM 39

CONDITION	RM 42-T5am16d	vs RM 43-T5am16d	(4)	RM 43-T5am16d	vs RM 43-T5wt	(1)
5 minutes after phage	180 \pm 23	*	296 \pm 67	328	-	-
10 minutes after phage	165 \pm 41	*	429 \pm 89	393	331	331
15 minutes after phage	212 \pm 27	*	361 \pm 50	225	-	-
20 minutes after phage	181 \pm 60	*	348 \pm 54	300	-	-
25 minutes after phage	157 \pm 45	*	320 \pm 69	213	212	212
30 minutes after phage	198 \pm 54		256 \pm 77	165	-	-
NaN ₃ + NaF + uninfected cells	19 \pm 7		19 \pm 10	-	-	-

CONDITION	RM 39-T5am16d	vs RM 43-T5am16d	(3)
5 minutes after phage	143 \pm 8	*	267 \pm 42
10 minutes after phage	165 \pm 21	*	405 \pm 92
15 minutes after phage	194 \pm 37	*	393 \pm 49
20 minutes after phage	259 \pm 72		352 \pm 66
25 minutes after phage	239 \pm 38		320 \pm 85
30 minutes after phage	234 \pm 21		283 \pm 70
NaN ₃ + NaF + uninfected cells	10 \pm 8		17 \pm 10

() indicates the number of experiments done in each group

* indicates a difference between the 2 samples with a confidence level, $p < 0.05$ [¹⁴C] α MG accumulation by infected cells was determined as outlined in MATERIALS AND METHODS. The results represent % of uptake, relative to that of uninfected control samples, in 90 seconds.

Fluorescence of Membrane-Bound NPN During T5A1⁻ Infections

When T5am16d infects RM 42, RM 43, or RM 39 in the presence of NPN, there is an increase in fluorescence intensity at about three minutes after infection but no increase at about 6 minutes (Fig.17). Since the mutant is deficient in second-step DNA transfer, this is consistent with Hantke and Braun's idea that the second increase in fluorescence intensity reflects membrane alterations related to second-step DNA transfer (41).

What is evident, however, is the marked increase in fluorescence intensity at about 10 to 12 minutes only occurring after infection of RM 43. This is a pattern similar to that found after T5 wild-type infection of the ColIb⁺ cells.

Potassium Efflux During T5am16d Infections of RM 43

Cells were preloaded with radiolabeled potassium ions as described above. As can be seen in Table 11 and Fig. 18, the pattern of ionic efflux in T5am16d-infected RM 43 is similar to that during T5 wild-type infection of RM 43. After plateauing early after infection, the numbers of residual intracellular counts falls rapidly beginning at about 8 to 10 minutes in each case. The reason for the greater early retention of preloaded ions in the T5 wild-type-infected cells is unknown. These figures are much higher than those obtained in other experiments (Table 6). Since the amount of initial efflux is very dependent upon multiplicity of infection (94) and since the data in Tables 6 and 11 were derived on different days, it might be that the multiplicities of infection were not comparable. If so, however, this was inadvertent. Nonetheless, if comparing the patterns of efflux seen

Figure 17. Fluorescence Intensity of N-phenyl-1-naphthylamine During T5A1⁻ Infections of RM 42, RM 43, and RM 39.

Cells were grown, concentrated, and infected in synthetic medium. At time zero, the infected cells were put into prewarmed growth medium containing the fluorescent probe. Relative fluorescence intensity was recorded in arbitrary units using a scanning fluorimeter.

- , RM 42
- , RM 43 (ColIb)
- ▲, RM 39 (ColIb h⁻)

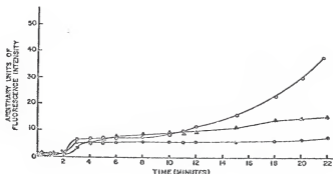


TABLE 11. Potassium Efflux from T5A1⁻-Infected RM 42 and RM 43

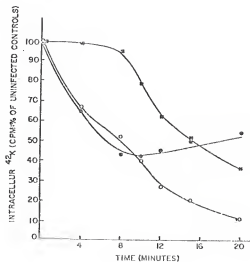
Minutes After Infection	RM 42-T5am16d	RM 43-T5am16d	RM 43-T5wt
4	65	67-55	99
8	43	52-48	95
10	42	40-33	79
12	46	27-29	62
15	51	21-17	51
20	55	11-9	37

RM 42, RM 43, and RM 39 were preloaded with ^{42}K . At intervals, uninfected cells were taken to determine the amount of potassium efflux. The results represent the amount of ^{42}K remaining inside infected cells, expressed as a % of the amount remaining in uninfected cells taken at the same time point. Leakage from infected RM 43 was measured during two trials.

Figure 18. Potassium Efflux from T5A1⁺-Infected RM 42 and RM 43.

Cells were grown in the presence of radiolabeled potassium, spun, washed, and infected or mock-infected. At time zero, the infected or mock-infected cells were resuspended in medium without the radiolabel. At the indicated intervals thereafter, 0.9 ml samples were removed, filtered over glass fiber filters, and the filters were washed. The amount of residual, intracellular labeled potassium was determined by liquid scintillation counting. The results represent the amount of ^{42}K remaining inside infected cells, expressed as a % of the amount remaining in mock-infected cells taken at the same time point.

- , T5am16d-infected RM 42
- , T5am16d-infected RM 43 (Col1b)
- , T5wt-infected RM 43 (Col1b)



during T5 wild-type or T5aml16d infection of RM 43 to the efflux seen after T5aml16d infection of RM 42, it is apparent that the initial decline in residual counts during infection of RM 42 plateaus at about 8 minutes after infection. This is not true during infection of RM 43, wherein ionic efflux is marked beyond 10 minutes after infection.

Absence of Host DNA Breakdown During T5aml16d or T5amlH27 Infection

To confirm the identity of T5aml16d and T5amlH27 mutants, a number of studies were performed. As can be seen in Table 1, these mutants do not plate efficiently unless the strain has suppressor activity. I have also tested these mutants for their ability to break down host DNA into acid-soluble fragments. This is the function of the A1 polypeptide, and should be lacking in A1⁻ mutants (62).

RM 42, RM 43, and RM 39 were grown in the presence of [³H]-thymidine as described in MATERIALS AND METHODS. As seen in Fig. 19, host DNA is not degraded to acid-soluble material after infection by T5A1⁻ mutants, confirming the lack of production of a functional A1 polypeptide.

Gel Analysis of Phage Proteins

It was suggested that RM 43 (Colib) could have an amber suppressor present on the plasmid, invalidating any conclusions regarding infection of these cells with amber mutants. To show that this was not the case, by confirming that class II (early) or class III (late) proteins are not made in A1⁻-infected, Colib⁺ cells, we pulse labeled proteins synthesized from 1 to 6 minutes, 6 to 11 minutes, or 11 to 16 minutes after T5aml16d infections of the colicinogenic hosts (RM 43). On SDS-polyacrylamide gels, we compared these samples with those of T5aml16d

Figure 19. Absence of Host DNA Breakdown During T5A1 Infections of RM 42, RM 43, and RM 39.

DNA was labeled by growing the cells in the presence of $[3H]$ thymidine ($0.4 \mu Ci$ per ml, $1 \mu Ci$ per $50 \mu g$ of thymidine). The cells were spun, concentrated, infected, and rediluted in medium lacking radiolabeled thymidine. At times thereafter, 0.9 ml samples were removed and placed in 0.1 ml of 50% TCA. Of this, 0.4 ml was filtered over glass fiber filters and washed. The numbers of acid-precipitable counts were determined by liquid-scintillation counting. In Fig. 19a, the host is RM 42; in Fig. 19b, the host is RM 43 (Colib), whereas in Fig. 19c, the host is RM 39 (Colib h^-).

- , uninfected cells
 ■, T5-infected cells
 ●, T5am16d-infected cells
 ▲, T5am127-infected cells

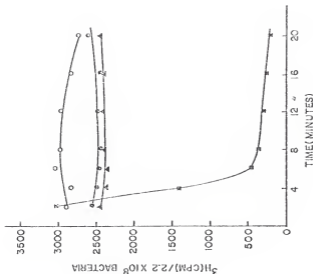


Figure 19a

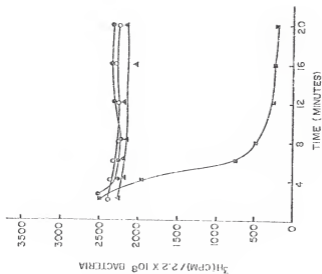


Figure 19b

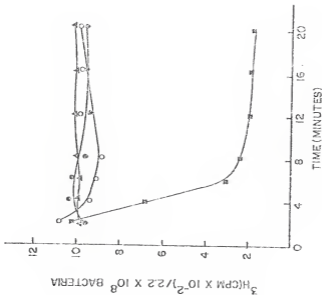


Figure 19c

infections of RM 42, and with T5 wild-type infections of both strains. Only class I (pre-early) proteins were seen during $A1^-$ infections of either host strain (Fig. 20).

Figure 20. Gel Analysis of Phage-Induced Proteins During T5 and T5A1⁻ Infections of RM 42 and RM 43.

Cells were grown and infected in a synthetic medium. At the times indicated, RM 42 or RM 43 (ColIb) infected with either T5 or T5am16d were labeled with 1 μ Ci per ml of a [¹⁴C] amino acid mixture. Chloramphenicol was added 5 minutes later, and the cells were chilled. Then, they were centrifuged, washed and resuspended in 1/10th volume of electrophoresis buffer and boiled for 5 minutes. Electrophoresis of 20 μ l samples was on a 15% slab gel for 14 hours at 75 volts. The dried gel was autoradiographed for 10 days by exposing it to Kodak XRI film.

The numbers across the bottom indicate the time after infection when the pulse began. The proteins were called pre-early or early according to their time of appearance (70).

Pre-early ===
 Early ---
 Early-----
 Pre-early ----

Early -----

Pre-early ----
 Pre-early ----
 Pre-early ----

RM 42				RM 43			
U	1	6	11	U	1	6	11
T5				T5			
A1 ⁻				A1 ⁻			

DISCUSSION

It has been known for more than 10 years that T5 is unable to replicate in hosts containing the colicin Ib factor (80, 97). Very little is known, however, about the mechanism whereby phage development is inhibited. Originally, no class II (early) or class III (late) proteins or RNA was seen on polyacrylamide gels. Because of this, it was hypothesized that a class-specific transcriptional block occurred (79). Later, though, it was found that some class II (early) proteins do actually appear. Additionally, functional RNA polymerase can be recovered from abortively infected cells (99), and no changes in the DNA template are found that explain the abortive infection (45, 89). Thus, there seems to be no evidence to implicate a primary transcriptional or translational dysfunction.

It seems more plausible to suggest that a generalized cellular dysfunction is the cause. During abortive infection, there is abrupt, simultaneous cessation of both transcription and translation, and there are numerous other concurrent physiological alterations not easily explained by specific-site inhibition. As membrane defects are thought to play a key role in other abortive systems, I decided to see if changes in membrane function during T5 infections of ColIb⁺ hosts could underlie the abortive process.

Previously, others in Donna Duckworth's lab have reported that uptake of thio- β -methyl-D-galactoside (TMG), a nonmetabolizable lactose analogue taken up by an ATP-dependent, active transport

mechanism (104) is inhibited during abortive infection (19). In the present study, I sought to further define the membrane's functional defects by looking at uptake of three additional substances -- proline, glutamine, and α MG -- each of which is taken up by a different mechanism (reviewed in 102).

Initially, however, I wanted to define the abortive system in our laboratory. In agreement with the results reported by others (79), incorporation of radiolabeled uridine into TCA-insoluble macromolecules was found to stop 9 to 12 minutes after T5 infection of cells containing the ColIb factor. This was the same time at which cessation of tyrosine and proline incorporation into acid-insoluble macromolecules occurred during abortive infection. Since I wished to define physiological alterations directly related to this abrupt interruption of the infectious process, I looked at changes occurring within the first 30 minutes after infection.

During abortive infection glutamine accumulation is inhibited, beginning to decline by 10 minutes after infection. Since the active transport of glutamine is an ATP-dependent process requiring periplasmic binding proteins (6, 7), decreased uptake ability might be due to decreased available ATP, loss of periplasmic binding proteins, or marked permeability changes which prevent concentration of the substance. The last of these explanations probably cannot totally explain the data, since at a time when glutamine accumulation is markedly depressed, α MG accumulation is three- to four-fold greater than that in uninfected cells. Regarding the loss of binding proteins, the inner membranes of abortively infected cells do not become sensitive

to SDS-induced lysis until more than 20 minutes after infection (19), so it is unlikely that the outer membrane integrity is destroyed to an extent necessary for periplasmic proteins to leak by 10 minutes after infection. The most likely explanation for the inhibition of glutamine uptake is that adequate energy is no longer available.

Net uptake of proline decreases during abortive infection at about the same time, or slightly later than the decline of glutamine accumulation. Proline uptake, unlike that of glutamine, does not require periplasmic binding proteins (6, 7). Even isolated membrane vesicles are capable of proline uptake, indicating that components of the uptake system are bound firmly to the membrane (reviewed in 102). Nonetheless, proline uptake is similar to glutamine uptake in that it requires an "energized membrane." Invoking the same argument against marked membrane permeability changes by 10 to 15 minutes after infection, I think the most likely reason for decreased proline accumulation is also membrane deenergization.

By membrane deenergization, I refer to loss of the transmembrane electrochemical gradient normally present (73). Since ions at low concentration obey the gas laws, this gradient can be expressed as:

$$\Delta \bar{\mu}_{H^+} = \Delta \psi - \frac{2.3RT}{F} \Delta pH,$$

where $\Delta \bar{\mu}_{H^+}$ equals the electrochemical proton gradient, $\Delta \psi$ represents electrical potential difference, R is the gas constant (8.3 joules per Kelvin degree), T equals the temperature in Kelvin degrees, F is the faraday (96,500 coulombs), and ΔpH means the proton gradient.

It is thought that a normal bacterium has a transmembrane electrical potential of 75 mV (84) to 140 mV (40), but at pH's near 7, ΔpH is

very small (84, 88). Therefore, under conditions used in the proline and glutamine uptake experiments, loss of membrane energization would be equivalent to loss of ionic polarization.

If decreases of proline and glutamine accumulation do indeed result from membrane deenergization during abortive infection, the prediction is that loss of ionic polarization should occur at the same time. This, in fact, has been shown herein, at least for the case of a major intracellular cation, potassium. Efflux of preloaded potassium from abortively infected cells becomes significantly greater than efflux from productively infected cells at about 10 minutes after infection.

Another prediction which is based on the assumption that the membrane is deenergized is that the fluorescence intensity omitted by NPN should increase dramatically during abortive infection. This change in fluorescence signal is observed when bacteria are treated with substances, such as derivatives of carbonyl cyanide phenylhydrazone, known to dissipate the high energy state of membranes (44). Since this lipophilic probe is neutral at physiological pH, it should not respond merely to changes in transmembrane potential (81). Rather, the rise in intensity is thought to occur due to structural changes in the outer membrane that are secondary to membranar deenergization (44). The structural changes in the outer membrane allow more NPN to bind to the inner membrane. Since the fluorescence intensity emitted by NPN increases directly as its environment becomes more hydrophobic (Leonard Rosenberg, personal communication), the observed increase is consistent with the interpretation that more of the probe moves into the lipid phase of the membrane. How or why it happens

was not investigated in the current study, but the predicted fluorescence changes did, in fact, coincide with the time when membrane depolarization is thought to occur in abortive infection.

Since binding-protein transport, represented by glutamine uptake, and membrane-bound transport, exemplified by proline uptake (reviewed in 102) were both inhibited, I decided to see if a substance taken up by group translocation was similarly affected. α MG is a nonmetabolizable glucose analogue taken into the cell by the phosphotransferase system (57). As the sugar is taken into the cell it is phosphorylated using phosphoenolpyruvate as its primary source of the phosphate group. At first, it seemed surprising that I found α MG accumulation was stimulated during abortive infection. However, this result is to be expected if the bacterial membrane becomes deenergized (Hans Kornberg, personal communication). In the absence of an energy-requiring dephosphorylating reaction, α MG becomes trapped within the cell as its phosphorylated derivative (49, 52). In fact, within a wide range of concentrations, dinitrophenol or azide causes a marked stimulation of α MG uptake (32).

In all cases, the pathophysiological alterations indicative of membrane depolarization occur only during abortive infection. When the infection is productive because of plasmid-borne or phage-borne mutations, uptake systems function as in normal T5 infections of ColIb⁻ hosts, and neither the pronounced potassium leakage nor the abnormal fluorescence pattern is seen. It appears likely, therefore, that the membrane alterations are due to genetic determinants on the phage genome or plasmid DNA at those loci which also produce cessation of the infectious process.

The abortive pattern of pathophysiological alterations occurs, however, when Col1b⁺ cells are infected with mutant phage deficient in second-step DNA transfer. The identity of the A1⁻ mutants used in our laboratory has been confirmed previously (29). I have also confirmed the mutant's inability to destroy the host genome, a known A1 function (62). Furthermore, gel analysis of proteins synthesized during A1⁻ infections confirms the absence of early and late proteins. These data are strong evidence that only pre-early phage genes are required for the abortive response. They also indicate that the shut-down of phage gene expression is not the result of a class-specific transcriptional or translational defect, since the cessation of macromolecular synthesis during abortive infection usually occurs after early gene expression has begun (45, also see Fig 20 of this dissertation). Stated another way, the mechanism operable in shutting off the T5 replicative cycle does so at 10 to 12 minutes after infection, regardless of whether pre-early or early proteins are being synthesized at that time.

The evidence against a specific-site block and the data indicative of membrane depolarization strongly suggest that the mechanism leading to the abortive response is quite similar to the killing action of phage ghosts and to the bactericidal action of colicin proteins E1, K, Ia, and Ib (66). Osmotically shocked T-even phage lose their DNA and their infectivity, but the empty protein shells retain the capacity to kill the host cell (47,48). Within two minutes, these "ghosts" inhibit DNA, RNA, and protein synthesis (27). Also quite early, uptake of DL-leucine and nucleic acid precursors (27), as well as the

uptake of β -galactosides (103), is diminished. Phosphorylated compounds including ATP, UTP, UDP, UMP, α -MG phosphate, and thio- β -methylgalactoside phosphate leak from ghost-treated cells (30,48). Yet σ -nitrophenylgalactoside (ONPG), carbamyl phosphate, or ATP entry is not enhanced (30) indicating a peculiar sort of one-way permeability defect. Additionally, ghost preparations contain some factor which causes a variable amount of cell lysis (47), but this lysis can be prevented by 0.05 M spermidine without influencing cell killing (D. Duckworth, unpublished data, cited in 28). Thus, phage ghosts inhibit macromolecular synthesis, inhibit active transport systems, cause "excretion" of at least some phosphorylated compounds, and cause a certain amount of cell lysis.

Similar actions are observed when sensitive bacteria are treated with colicin E1, K, Ia, or Ib -- collectively representing a group of proteins which are produced by plasmid-containing Enterobacteria and which seem to kill bacteria by the same (or similar) mechanism(s) (reviewed in 64). These proteins, for example, inhibit DNA, RNA, and protein synthesis (63,82). They also inhibit uptake of several amino acids (Gilchrist & Konisky, unpublished data, cited in 55,65; J. P. Kabat, cited in 86), of β -galactosides (36, Gilchrist and Konisky, unpublished data cited in 55), and of potassium (Gilchrist & Konisky, unpublished data, cited in 55,101), rubidium (101), magnesium, and cobaltous ions (67). Colicins accelerate loss of preaccumulated potassium (Gilchrist & Konisky, unpublished data, cited in 55,101), magnesium, and cobalt ions (67) -- a change unlike that due to energy poisons (93) -- probably indicating a permeability defect. Also,

they cause cells to lose phosphorylated compounds (37), sugars, and amino acids (30, Gilchrist & Konisky, unpublished data, cited in 55). The membrane lipid and protein composition of treated cells is altered (16,17,18,53,90) and, under some conditions, the cells lyse (17). The lysis does not occur in minimal medium (67). Further, the rate of ONPG hydrolysis is unchanged (36), ATP is not found free in the medium (35), and α -MG uptake is augmented (52). Therefore, permeability to, or excretion of, various substances seems to be selective. In summary, physiological changes induced by ghosts and by colicins E1, K, Ia, and Ib are quite similar.

This group of colicins is thought to act by forming ion channels (91). The evidence for this is derived from in vitro studies with phospholipid bilayers. Voltage-dependent, ion-permeable channels are introduced into bilayers by purified colicin preparations. Other data are consistent with this proposal. (1) Fluorescent dye probes indicate membrane deenergization (44,85), and the electrical transmembrane gradient is dissipated (100). (2) The decreased ATP level, characteristic after colicin treatment, is prevented in colicin E1- or colicin K-treated cells by chemically or genetically inducing loss of membrane-bound ATPase activity (86). The inference is that the ATPase-deficient cells are unable to exhaust ATP supplies in what would be a futile effort to reestablish membrane polarity. (3) When ATP supplies are maintained in this way, macromolecular synthesis continues at a substantial rate (75), but transport activities are not improved. The idea arising from these observations is that cessation of macromolecular synthesis is secondary to the ATP deficit. However,

the membrane depolarization is not corrected, and therefore, active transport systems are still impaired. (4) ATP'ase-deficient strains, when grown in glucose medium with high Mg^{++} and K^+ concentrations, remain viable in the presence of colicin K or E1 (56). Under these conditions, essential intracellular cation concentrations are maintained, and the cell can continue metabolic functions. Since physiological studies completed thus far indicate numerous similarities among abortive T5 infections, phage ghost action, and colicin action, it might be that they all act by a mechanism somewhat similar to this.

Recently it has been proposed that T5 and BF23 infectious processes halted by the colicin Ib protein itself (D. J. McCorquodale, *et al.*, submitted to J. Virol.; D. J. McCorquodale, personal communication). According to the model, the pre-early A3 gene product inactivates an immunity protein, which normally protects the bacterium from the colicin it produces. Abortive infection should not occur if the ColIb plasmid is mutated such that the colicin protein is not produced, nor should it occur if the immunity protein is altered in such a way that it cannot be bound by the A3 protein but that it can still prevent the bactericidal action of the colicin protein. The infectious process should likewise proceed unhindered if there are alterations at the target site of the colicin protein or in biochemical pathways required for expression of the colicin's lethality. Furthermore, according to the model, other abortive systems such as T7 infection of F-containing *E. coli* and T4rII mutant infection of λ lysogens can be similarly explained. In each case, the hypothesis is that a potentially lethal protein encoded on the extrachromosomal

element (42) is activated by the infection -- leading to the cessation of the infectious process before progeny appear.

This model, though promising, is currently based only on circumstantial evidence. Although an immunity protein for colicin E3 has been isolated (51), no direct evidence for the existence of a colicin Ib immunity protein has been described. Secondly, some permissive hosts bearing plasmid mutations have been found to lack the ability to produce the colicin protein (S. S. Tung, cited by D. J. McCorquodale), while other permissive strains with mutant plasmids do produce colicin (D. J. McCorquodale, personal communication). This does suggest that both the colicin and an immunity protein are involved in abortive infections, but the mutant strains have not been characterized well enough to say this with any certainty. Thirdly, there is only a weak suggestion that the chromosomal loci, cmrA and cmrB, involved in abortive infection are also the loci coding for the colicin Ib target. The evidence cited is that cmrA and cmrB map close to trkA and trkB, loci involved in potassium transport (33). Also, the tolI gene, which confers tolerance to colicin Ib (15), maps close to another locus, trkC, involved in potassium transport (33). From these data, the hypothesis is that the cmrA, cmrB, and tolI loci are identical to trkA, trkB, and trkC, respectively. Beyond this, it must be assumed that the potassium transport system is the target of colicin Ib. Fourthly, the comparison of the ColIb system with other abortive infections cannot be as direct as is suggested by the model. For example, in the T4rII mutant system, the lack of a functional rII protein contributes to the abortive infection, whereas the presence of the A3 gene product is necessary for abortive T5 infection.

Nonetheless, the model is useful as a working hypothesis, as many testable predictions can be made from it. It should be possible to isolate a colicin Ib immunity protein, for example. Further, it should be possible to demonstrate an immunity protein-colicin Ib complex, to which the A3 phage gene product should bind. The addition of the A3 gene product should release a form of colicin Ib lethal for strains normally immune to, but not resistant to, the bactericidal action. Also, the prediction is that cmrA⁻ and cmrB⁻ mutants should be resistant to colicin Ib and should have defects in potassium transport. Importantly, strains with trkA, trkB, and tolI mutations which have received the ColIb plasmid should be permissive hosts for T5. Finally, it might be possible to find ionic conditions or membrane-stabilizing conditions under which abortive infection is prevented. Thus far, however, I have unsuccessfully tried to circumvent abortive infection using high concentrations of potassium, magnesium, calcium, alone or in combination; stabilizing the membrane with sucrose or polyamines have similarly failed to affect abortion. It is interesting in this regard, however, that ATPase inhibition, combined with high cation concentrations in the medium, was ineffective in preventing colicin Ib-induced cell death (56).

Another approach which holds great promise to understanding the role of the plasmid is restriction fragment analysis (64,95). EcoRI fragments of ColIb, for example, could be sized and ordered. Then, individual fragments could be spliced to carrier plasmids, and the chimerae placed into suitable ColIb⁻ strains. The functions of the various fragments could then be ascertained. A modification of this

would be to determine the ability of the various chimeric plasmids to complement Col1b factor mutants. In this way, genes controlling particular functions can be mapped.

In summary, evidence has been shown which indicates that membrane depolarization occurs at about the same as cessation of macromolecular synthesis during abortive T5 infection. Only the pre-early phage genes are necessary to elicit the same pattern of responses. No conclusive evidence exists to link the membrane depolarization and the interruption of the infectious process in a cause-effect relationship. Nonetheless, the results do suggest a marked similarity among abortive T5 infection, the cell killing of phage ghosts, and the bactericidal action of certain colicin proteins. The data presented here are consistent with a recently proposed model, which hypothesizes that the colicin 1b protein is in fact directly responsible for the abortive infection.

BIBLIOGRAPHY

1. Adams, M. H. 1959. Bacteriophages. Interscience Publications, Inc., New York, N.Y.
2. Anderson, C. W., and J. Eigner. 1971. Breakdown and exclusion of superinfecting T-even bacteriophage in Escherichia coli. J. Virol. 8:869-886.
3. Anderson, C. W., J. R. Williamson, and J. Eigner. 1971. Localization of parental deoxyribonucleic acid from superinfecting T4 bacteriophage in Escherichia coli. J. Virol. 8:887-893.
4. Bauer, W., and J. Vinograd. 1968. The interaction of closed circular DNA with intercalative dyes. I. The superhelix density of SV40 DNA in the presence and absence of dye. J. Mol. Biol. 33:141-171.
5. Beckman, L. D., G. C. Anderson, and D. J. McCorquodale. 1973. Arrangement on the chromosome of the known pre-early genes of bacteriophage T5 and 8F23. J. Virol. 12:1191-1194.
6. Berger, E. A. 1973. Different mechanisms of energy coupling for the active transport of proline and glutamine in Escherichia coli. Proc. Nat. Acad. Sci. 70:1514-1518.
7. Berger, E. A., and L. A. Heppel. 1974. Different mechanisms of energy coupling for the shock-sensitive and shock-resistant amino acid permeases of Escherichia coli. J. Biol. Chem. 249:7747-7755.
8. Bertani, G. 1958. Lysogeny. Adv. Virus Res. 5:151-193.
9. Bertani, L. E., and G. Bertani. 1971. Genetics of P2 and related phages. Adv. Genetics 16:199-237.
10. Bertani, L. E., and J. A. Levy. 1964. Conversion of lysogenic E. coli by nonmultiplying superinfecting bacteriophage P2. Virology 22:634-640.
11. Blumberg, D. E., C. J. Mabie, and M. H. Malamy. 1976. T7 protein synthesis in F-factor-containing cells: Evidence for an episonally induced impairment of translation and the relation to an alteration in membrane permeability. J. Virol. 17:94-105.

12. Britton, J. R., and R. Haselkorn. 1975. Permeability lesions in male *Escherichia coli* infected with bacteriophage T7. *Proc. Nat. Acad. Sci.* 72:2222-2226.
13. Britton, J. R., and R. Haselkorn. 1975. Macromolecular synthesis in T7 infected F' cells. *Virology* 67:264-275.
14. Buller, C. S., and L. Astrachan. 1968. The replication of T4 rII bacteriophage in *Escherichia coli* K-12 (λ). *J. Virol.* 2:298-307.
15. Cardelli, J., and J. Konisky. 1974. Isolation and characterization of an *Escherichia coli* mutant tolerant to colicins Ia and Ib. *J. Bacteriol.* 119:379-385.
16. Cavard, D., C. Rampini, E. Barbu, and J. Polonovski. 1968. Activate' phospholipasique et autres modifications du metabolisme des phospholipides consecutives à l'action des colicines sur *E. coli*. *Bull. Soc. Chem. Biol.* 50:1455-1471.
17. Cavard, D., J. Schirmann, M. Lux, C. Rampini, and E. Barbu. 1967. Action lytique des colicines. *C. R. Acad. Sci.* 265:1255-1257.
18. Cavard, O., M. Vallee, and E. Barbu. 1974. Mise en evidence par le lauryl-sulfate de quelques modifications precoces de la membrane de *E. coli* provoquées par les phages T4, les fantômes de phages T4 et la colicine K. *Biochimie* 56:221-230.
19. Cheung, A. K., and O. H. Duckworth. 1977. Membrane damage and the abortive infection of ColIb-containing *E. coli* by bacteriophage T4. *J. Virol.* 23:98-105.
20. Clewell, O. B., and O. R. Helinski. 1969. Supercoiled circular DNA protein complex in *Escherichia coli*: purification and induced conversion to an open circular DNA form. *Proc. Nat. Acad. Sci.* 62:1159-1166.
21. Clewell, O. B., and O. R. Helinski. 1970. Existence of the colicinogenic factor-sex factor ColIb-P9 as a supercoiled circular DNA-protein relaxation complex. *Biochem. Biophys. Res. Comm.* 41:150-156.
22. Cohen, S. N., F. Cabello, M. Casadaban, A. C. Y. Chang, and T. Kimmis. 1977. DNA cloning and plasmid biology. In: M. A. Scott and R. Werner (eds.). *Molecular cloning of recombinant DNA*, Academic Press, New York.
23. Condit, R. C. 1975. F factor mediated inhibition of bacteriophage growth: increased membrane permeability and decreased ATP levels following T7 infection. *J. Molec. Biol.* 98:45-56.

24. Condit, R. C., and J. A. Steitz. 1975. F factor-mediated inhibition of bacteriophage T7 growth: Analysis of T7 RNA and protein synthesis in vivo and in vitro using male and female Escherichia coli. J. Mol. Biol. 98:31-43.
25. Crawford, L. V. 1959. Nucleic acid metabolism in Escherichia coli infected with phage T5. Virology 7:359-374.
26. Dove, W. 1971. In: A. D. Hershey (ed.), The bacteriophage lambda. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. Duckworth, D. H. 1970. The metabolism of T4 phage ghost infected cells. I. Macromolecular syntheses and transport of nucleic acid and protein precursors. Virology 40:673-684.
28. Duckworth, D. H. 1970. Biological activity of bacteriophage ghosts and "take-over" of host functions by bacteriophage. Bacteriol. Rev. 34:344-363.
29. Duckworth, D. H., G. B. Dunn, and D. J. McCorquodale. 1976. Identification of the gene controlling the synthesis of the major bacteriophage T5 membrane protein. J. Virol. 18:542-549.
30. Duckworth, D. H., and H. H. Winkler. 1972. Metabolism of T4 bacteriophage ghost-infected cells. II. Do ghosts cause a generalized permeability change? J. Virol. 9:917-922.
31. Dulbecco, R. 1952. Mutual exclusion between related phages. J. Bacteriol. 63:209-217.
32. Englesberg, E., J. A. Watson, and P. A. Hoffee. 1961. The glucose effect and the relationship between glucose permease, acid phosphatase, and glucose resistance. Cold Spring Harbor Symposium Quant. Biol. 26:261-276.
33. Epstein, W., and B. S. Kim. 1971. Potassium transport loci in Escherichia coli K-12. J. Bacteriol. 108:639-644.
34. FerroLuzzi-Ames, G., and B. N. Ames. 1965. The multiplication of T4rII phage in E. coli K-12 (λ) in the presence of polyamines. Biochem. Biophys. Res. Comm. 18:639-647.
35. Fields, K. L. 1969. Comparison of the action of colicins E1 and K on E. coli with the effects of abortive infection by virulent bacteriophages. J. Bacteriol. 97:78-82.
36. Fields, K. L., and S. E. Luria. 1969. Effects of colicins E1 and K on transport systems. J. Bacteriol. 97:57-63.
37. Fields, K. L., and S. E. Luria. 1969. Effects of colicins E1 and K on cellular metabolism. J. Bacteriol. 97:64-77.

38. Fredericq, P. 1957. Colicins. *Ann. Rev. Microbiol.* 11:7-22.
39. Garen, A. 1961. Physiological effects of rII mutations in bacteriophage T4. *Virology* 14:151-163.
40. Griniuvienė, B., V. Chmielaskaitė, and L. Grinius. 1974. Energy-linked transport of permeant ions in *Escherichia coli* cells: evidence for membrane potential generation by proton-pump. *Biochem. Biophys. Res. Comm.* 56:206-213.
41. Hantke, K., and V. Braun. 1974. Fluorescence studies on first steps of phage-host interactions. *Virology* 58:310-312.
42. Hardy, K. G. 1975. Colicinogeny and related phenomena. *Bacteriol. Rev.* 39:464-515.
43. Harold, F. M., and J. R. Baarda. 1969. Inhibition of membrane-bound adenosine triphosphatase and cation transport in *Streptococcus faecalis* by NH' -dicyclohexyl-carbodiimide. *J. Biol. Chem.* 224:2261-2269.
44. Helgerson, S. L., and W. A. Cramer. 1976. Changes in *E. coli* cell envelope structure caused by uncouplers of active transport and colicin E1. *J. Supramolecular Structure* 5:291-308.
45. Herman, R. C., and R. Moyer. 1975. *In vivo* repair of bacteriophage T5 DNA: an assay for viral growth control. *Virology* 66:393-407.
46. Herriott, R. M., and J. L. Barlow. 1952. Preparation, purification, and properties of *E. coli* virus T2. *J. Gen. Physiol.* 36:17-28.
47. Herriott, R. M., and J. L. Barlow. 1957. The protein coats or "ghosts" of coliphage T2. I. Preparations, assay, and some chemical properties. *J. Gen. Physiol.* 40:809-825.
48. Herriott, R. M., and J. L. Barlow. 1957. The protein coats or "ghosts" of coliphage T2. II. The biological functions. *J. Gen. Physiol.* 41:307-331.
49. Hoffee, P., E. Englesberg, and F. Lamy. 1964. The glucose permease system in bacteria. *Biochim. Biophys. Acta* 79: 337-350.
50. Hull, R., and E. E. Moody. 1976. Isolation and genetic characterization of *Escherichia coli* K-12 mutations affecting bacteriophage T5 restriction by the ColIb plasmid. *J. Bacteriol.* 127:229-236.
51. Jakes, K. S., and N. D. Zinder. 1974. Highly purified colicin E3 contains immunity protein. *Proc. Nat. Acad. Sci.* 71:3380-3384.

52. Jetten, A. M. 1976. Effects of colicins K and E1 on the glucose phosphotransferase system. *Biochim. Biophys. Acta* 440:403-411.
53. Knepper, J. E., and J. E. Lusk. 1976. Disappearance of specific proteins from the membranes of colicin-treated cells. *J. Biol. Chem.* 251:7577-7580.
54. Knopf, K-W., and H. Bujard. 1975. Structure and function of the genome of coliphage T5. Transcription *in vitro* of the "nicked" and "nick-free" T5 DNA. *Eur. J. Biochem.* 53:371-377.
55. Konisky, J. 1975. Interaction of colicin Ia with *Escherichia coli*. In: D. Schlessinger (ed.), *Microbiology-1974*. American Society for Microbiology, Washington, D.C.
56. Kopecky, A. L., D. P. Copeland, and J. E. Lusk. 1975. Viability of *Escherichia coli* treated with colicin K. *Proc. Nat. Acad. Sci.* 72:4631-4634.
57. Kundig, W., S. Ghosh, and S. Roseman. 1964. Phosphate bound to histidine in a protein as an intermediate in a novel phosphotransferase system. *Proc. Nat. Acad. Sci.* 52:1067-1074.
58. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227:680-685.
59. Lanni, Y. T. 1960. Invasion by bacteriophage T5. II. Dissociation of calcium-independent and calcium-dependent processes. *Virology* 10:514-529.
60. Lanni, Y. T. 1965. DNA transfer from phage T5 to host cells: dependence on intercurrent protein synthesis. *Proc. Nat. Acad. Sci.* 53:969-973.
61. Lanni, Y. T. 1968. First-step-transfer deoxyribonucleic acid of bacteriophage T5. *Bacteriol. Rev.* 32:227-242.
62. Lanni, Y. T. 1969. Functions of two genes in the first-step-transfer DNA of bacteriophage T5. *J. Mol. Biol.* 44:173-183.
63. Levinsohn, R., J. Konisky, and M. Nomura. 1967. Interaction of colicins with bacterial cells. IV. Immunity breakdown studies with colicins Ia and Ib. *J. Bacteriol.* 96:811-821.
64. Lewin, B. 1977. Gene expression. Vol. 3, Plasmids and phages. John Wiley and Sons, New York, N.Y.
65. Luria, S. E. 1964. On the mechanism of action of colicins. *Ann. Inst. Pasteur Paris* 107:67-73.
66. Luria, S. E. 1975. Colicins and the energetics of cell membranes. *Scien. Am.* 233:30-37.

67. Lusk, J. E., and D. L. Nelson. 1972. Effects of colicins E1 and K on permeability to magnesium and cobaltous ions. *J. Bacteriol.* 112:148-160.
68. Mäkelä, O., P. H. Mäkelä, and S. Soikkeli. 1964. Sex-specificity of the bacteriophage T7. *Ann. Med. Exp. Fenn.* 42:188-195.
69. McCorquodale, D. J. 1975. The T-odd bacteriophages. *C.R.C. Crit. Rev. Microbiol.* 4:101-159.
70. McCorquodale, D. J., and J. M. Buchanan. 1968. Patterns of protein synthesis in T5-infected *Escherichia coli*. *J. Biol. Chem.* 243:2550-2559.
71. McCorquodale, D. J., and Y. T. Lanni. 1970. Patterns of protein synthesis in *Escherichia coli* infected by amber mutants in the first-step-transfer DNA of T5. *J. Mol. Biol.* 48:133-143.
72. Meselson, M., R. Yuan, and J. Heywood. 1972. Restriction and modification of DNA. *Ann. Rev. Biochem.* 41:447-466.
73. Mitchell, P. 1966. Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Cambridge Philos. Soc.* 41:445-502.
74. Mizobuchi, K., G. C. Anderson, and D. J. McCorquodale. 1971. Abortive infection of bacteriophage BF23 due to the colicin Ib factor. I. Genetic studies of nonrestricted and amber mutants of bacteriophage BF23. *Genetics* 68:323-340.
75. Mizobuchi, K., and D. J. McCorquodale. 1974. Abortive infection by bacteriophage BF23 due to the colicin Ib factor. II. Involvement of pre-early proteins. *J. Mol. Biol.* 85:67-74.
76. Morrison, T. G., and M. H. Malamy. 1971. T7 translational control mechanisms and their inhibition by F factors. *Nature New Biol.* 231:37-41.
77. Moyer, R. W., and J. M. Buchanan. 1970. Effect of calcium ions on synthesis of T5-specific proteins. *J. Biol. Chem.* 245:5897-5903.
78. Moyer, R. W., and J. M. Buchanan. 1970. Effect on calcium ions on synthesis of T5-specific ribonucleic acid. *J. Biol. Chem.* 245:5904-5913.
79. Moyer, R. W., A. S. Fu, and C. Szabo. 1972. Regulation of bacteriophage T5 development by Col 1 factors. *J. Virol.* 9:804-812.
80. Nisioka, T., and H. Ozeki. 1968. Early abortive lysis by phage BF23 in *E. coli* K-12 carrying the colicin Ib factor. *J. Virol.* 2:1249-1254.

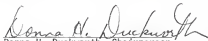
81. Nivea-Gomez, D., J. Konisky, and R. B. Gennis. 1976. Membrane changes in Escherichia coli induced by colicin Ia and agents known to disrupt energy transduction. Biochemistry 15:2747-2753.
82. Nomura, M. 1963. Mode of action of colicines. Cold Spring Harbor Symposium Quant. Biol. 28:315-324.
83. Novick, R. P. 1974. Bacterial plasmids. In: A. L. Laskin and H. A. Lechevalier (eds.), Handbook of microbiology, vol. IV. CRC Press, Cleveland, Ohio.
84. Padan, E., D. Zilberstein, and H. Rottenberg. 1976. The proton electrochemical gradient in Escherichia coli cells. Eur. J. Biochem. 63:533-541.
85. Phillips, S. K., and W. A. Cramer. 1973. Properties of the fluorescence probe response associated with the transmission mechanism of colicin E1. Biochemistry 12:1170-1176.
86. Plate, C. A., J. L. Suit, A. M. Jetten, and S. E. Luria. 1974. Effects of colicin K on a mutant of Escherichia coli deficient in Ca^{++} , Mg^{++} -activated adenosine triphosphatase. J. Biol. Chem. 249:6138-6143.
87. Rabussay, D., and E. P. Geiduschek. 1977. Regulation of gene action in the development of lytic bacteriophages. In: H. Fraenkel-Conrat and R. R. Wagner (eds.), Comprehensive virology, vol. 8. Regulation and genetics - Bacterial DNA viruses. Plenum Press, New York.
88. Ramos, S., and H. R. Kaback. 1977. The electrochemical proton gradient in Escherichia coli membrane vesicles. Biochemistry 16:848-854.
89. Reanney, D. 1976. Extrachromosomal elements as possible agents of adaptation and development. Bacteriol. Rev. 40:552-590.
90. Sabet, S. F. 1978. Colicins and bacterial membranes: structures and functions. J. Biol. Chem. 253:982-989.
91. Schein, S. J., B. L. Kagan, and A. Finkelstein. 1978. Colicin K acts by forming voltage-dependent channels in phospholipid bilayer membranes. Nature 276:159-163.
92. Sekiguchi, M. 1966. Studies on the physiological defect in rII mutants of bacteriophage T4. J. Mol. Biol. 16:503-522.
93. Silver, S. 1969. Active transport of magnesium in Escherichia coli. Proc. Nat. Acad. Sci. 62:764-772.
94. Silver, S., E. Levine, and P. M. Spielman. 1968. Cation fluxes and permeability changes accompanying bacteriophage infection of E. coli. J. Virol. 2:763-771.

95. Skurray, R. A., H. Hagiowski, and A. J. Clark. 1976. Molecular cloning of DNA from F sex factor of Escherichia coli K-12. Proc. Nat. Acad. Sci. U. S. 73:64-68.
96. Snedecor, G. W., and W. G. Cochran. 1967. Statistical methods, 6th ed.. Iowa State University Press, Ames, Iowa.
97. Strobel, M., and M. Nomura. 1966. Restriction of the growth of bacteriophage BF23 by a colicine I (ColI-P9) factor. Virology 28:763-765.
98. Szabo, C., B. Dharmgongartama, and R. W. Moyer. 1975. The regulation of transcription in bacteriophage T5-infected Escherichia coli. Biochemistry 14:989-996.
99. Szabo, C., and R. W. Moyer. 1975. Purification and properties of a bacteriophage T5-modified form of Escherichia coli RNA polymerase. J. Virol. 15:1042-1046.
100. Tokuda, H., and J. Konisky. 1978. Mode of action of colicin Ia: effect of colicin on the Escherichia coli proton electrochemical gradient. Proc. Nat. Acad. Sci. 75:2579-2583.
101. Wendt, L. 1970. Mechanism of colicin action: early events. J. Bacteriol. 104:1236-1241.
102. Wilson, D. B. 1978. Cellular transport mechanisms. Ann. Rev. Biochem. 47:933-965.
103. Winkler, H. H., and D. H. Duckworth. 1971. Metabolism of T4 bacteriophage ghost-infected cells: effect of bacteriophage and ghosts on the uptake of carbohydrates in Escherichia coli B. J. Bacteriol. 107:259-267.
104. Winkler, H. H., and T. H. Wilson. 1966. The role of energy coupling in the transport of β -galactosides by Escherichia coli. J. Biol. Chem. 241:2200-2211.
105. Yamamoto, K. R., B. M. Alberts, R. Benzinger, L. Lawhorne, and G. Treiber. 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology 40:734-744.

BIOGRAPHICAL SKETCH

Jerry Glenn was born on January 13, 1951, in Spangler, Pennsylvania. He attended public schools in that state and was graduated from Bellefonte High School in 1968. Thereafter, he enrolled in a five-year cooperative program in medicine offered jointly by The Pennsylvania State University and Jefferson Medical College of Philadelphia. The Bachelor of Science degree was awarded to him by Penn State in 1971, and the Doctor of Medicine degree was granted by Jefferson in 1973. In July of 1973, he began surgical training at the University of Florida Medical Center. He started graduate work in the Department of Immunology and Medical Microbiology two years later, in 1975, and received a Research Service Award (5 F32 AI-05296) from the National Institute of Allergy and Infectious Diseases in 1976. He married Judith McKean on December 14, 1978. After receiving his Ph.D. in June of 1979, he resumed surgical training at the University of Florida.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Donna H. Duckworth, Chairperson
Associate Professor of
Immunology and Medical
Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



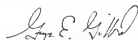
Kenneth I. Berns
Professor of Immunology
and Medical Microbiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Carl M. Feldherr
Associate Professor of
Anatomy

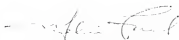
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



George E. Gifford
Professor of Immunology
and Medical Microbiology

This dissertation was submitted to the Graduate Faculty of the College of Medicine and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June 1979



Dean, College of Medicine



Dean, Graduate School



UNIVERSITY OF FLORIDA

3 1262 08554 6021